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INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR99/01715	13 July 1999	15 July 1998

TITLE OF INVENTION

ISOFLAVANOID-BASED THERAPEUTIC COMPOSITION INTENDED TO BE USED IN THE TREATMENT OF TUMOURS WITH CYTOTOXIC AGENTS

APPLICANT(S) FOR DO/EO/US

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Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for Internatl. Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the Internatl. Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

    International Search Report — EPO
    PCT Request Form
    PCT/IB/304 Form
    First Page of Publication
    International Preliminary Examination Report — NO annexes

US APPLICATION NO (If known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
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	CALCULATIONS	PTO USE ONLY
17. ☒ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Internatl. prelim. examination fee paid to USPTO (37 CFR 1.492 (a) (1)) . . $690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (2)) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) . . $710.00		
Neither international preliminary examination fee (37 CFR 1.492 (a) (3)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO) **$1000.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (4)) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
Search Report prepared by the EPO or JPO (37 CFR 1.492 (a) (5)) **$860.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of $130.00 for furnishing the **oath or declaration** later than ☐ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	**Number Filed**	**Number Extra**	Rate		
Total Claims	**8** - 20 =	-0-	x $18.00	$	
Independent Claims	**4** - 3 =	-1-	x $80.00	$ 80.00	
Multiple Dependent Claim(s) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 940.00	
Reduction by 1/2 for filing by **small entity**, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 940.00	
Processing fee of $130 for furnishing the **English translation** later than ☐ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(f))				$	
TOTAL NATIONAL FEE =				$ 940.00	
Fee of $40.00 for recording the enclosed **assignment** (37 CFR 1.21(h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31).				$	
TOTAL FEES ENCLOSED =				$ 940.00	
				Amt. to be refunded:	$
				Amt. charged:	$

a. ☒ A check in the amount of $ 940.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. **06-1358** in the amount of $ ____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge my account any additional fees set forth in §1.492 during the pendency of this application, or credit any overpayment to Deposit Account No. **06-1358**. A duplicate copy of this sheet is enclosed.

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JPH&S 3/95

ISOFLAVONOID-BASED THERAPEUTIC COMPOSITION INTENDED TO BE USED IN THE TREATMENT OF TUMOURS WITH CYTOTOXIC AGENTS

The present invention relates to the use of compounds of the isoflavonoid type in the treatment of cancers with cytotoxic agents.

A cancer is a disorder of the somatic genes in which genetic dysfunctions become amplified as the tumour process progresses from the state of a precancerous lesion to that of a malignant transformation, the cancerous tumour becoming metastatic and often resistant to cytotoxic medicaments.

In spite of major efforts made in all developed countries, in particular through experimental and clinical research programmes, mortality due to the various cancers (solid tumours and haematological neoplasias) remains unacceptably high. In many countries, the mortality caused by cancer is ranked second, just after cardiovascular diseases.

In terms of newly diagnosed cancers, the distribution between solid tumours and haematological neoplasias (bone marrow, blood, lymphatic system) shows that 9 cancers out of 10 are solid tumours. Contrary to what is observed in haematological oncology (therapeutic success in 40 to 90% of the cancers of the blood cells), only a small number of advanced or disseminated solid tumours respond to chemotherapy treatments alone. It is partly for this reason that the overall mortality caused by cancer increased in the USA between 1973 and 1992.

It is unfortunately not certain that this trend can be reversed solely by the appearance, besides the established chemotherapy arsenal, of novel antitumour medicaments such as taxanes (paclitaxel and docetaxel) which interfere with the formation of the microtubules (W.P. McGuire et al., Am. Intern. Med., 1989), the inhibitors of topoisomerases I derived from camptothecin (topotecan and irinotecan), vinorelbine

(novel alkaloid derived from periwinkle), gemcitabine (novel cytotoxic antimetabolic agent), raltitrexed (inhibitor of thymidylate synthetase) and miltefosine (first representative of the alkylphosphocholine family). These treatments are in addition, either as a first line treatment, or as a second line treatment, to the medicaments whose specific activity is now well recognized such as doxorubicin, cisplatin, vincristine, methotrexate, 5-fluorouracil.

One of the most difficult current problems of anticancer chemotherapy is due to the fact that many populations of malignant cells exhibit substantial resistance to the established cytotoxic substances. Most often, this situation results from the existence of multiresistance genes or from the frequency of genetic mutations in certain types of tumours. Thus, the treatment of cancers requires novel approaches, complementary to those currently used, and intended for better combating the extension and heterogeneity of the tumour load and the acquisition of "multi-cytotoxic drug" resistance.

Among these novel approaches, some are already promising. That is the case for the induction of apoptosis, the inhibition of tumour angiogenesis and of metastatic processes, not to mention gene therapy or immunotherapy.

The inventors were interested in a different approach. The objective sought was to make the population of tumour cells more sensitive to the reference anticancer treatments in order to achieve a double beneficial effect:

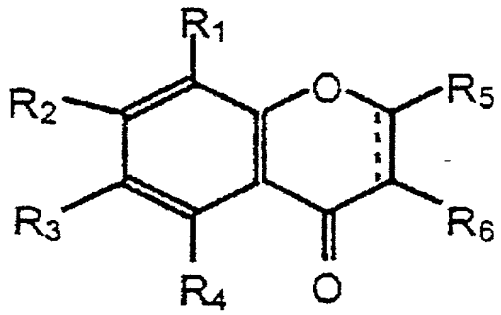
1) to increase the cytotoxic activity and therefore the efficacy, and

2) to reduce the frequency and the severity of certain side effects by virtue of the reduction of the dosage which might follow the induction of the increase in the antitumour efficacy.

It is this strategy which is at the origin of the discovery of an innovative mechanism caused by

substances having a low antitumour power or even lacking this power, but capable of inducing a very significant increase in the cytotoxic activity of proven anticancer medicaments. This innovative mechanism results from the possibility for these substances either to stimulate the recruitment of clonogenic cells inside the tumour, making it more sensitive to conventional treatment with cytotoxic agents, or to inhibit the proliferation of clonogenic cells, thus contributing to the regression of the tumour.

The subject of the present invention is thus the use, in the treatment of cancers with at least one antitumour agent chosen from cytotoxic agents, of a compound having an activity on the proliferation of clonogenic cells, chosen from isoflavonoids and analogous compounds of the chromone type and in particular the compounds of formula:



(I)

in which formula:

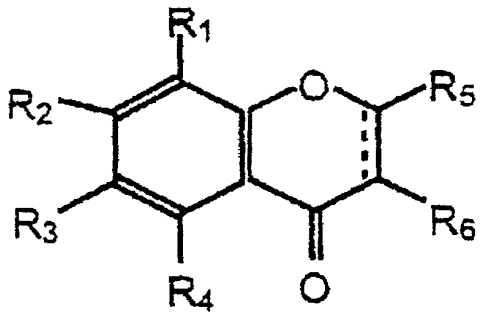
- R_1, R_2, R_3 and R_4 are chosen, independently of each other, from H, OH, a C_1-C_4 alkoxy group, an $-OCOR_7$ group, R_7 being a C_1-C_4 alkyl group, at least one of the substituents R_1, R_2, R_3 or R_4 being other than H and it being possible for R_2 and R_3 to form together a methylenedioxy group,
- R_5 is chosen from H, OH, a C_1-C_4 alkoxy group, an O-glycosyl group and a cyclohexyl group,
- R_6 is chosen from a cyclohexyl group, a phenyl group and a phenyl group substituted 1 to 3 times with groups chosen from H, OH and a C_1-C_4 alkoxy group,

- and ⎯⎯ denotes either a double bond, or a single bond.

A preferred class of compounds of formula I are those in which R_6 is chosen from the phenyl group, the 4-hydroxyphenyl group and the 4-(C_1-C_4alkoxy)phenyl groups.

The cytotoxic agents may be used at their usual dose and, in this case, their efficacy is enhanced, or at lower doses taking into account the increase in their antitumour efficacy if the desired objective is first to enhance the patient's tolerance to the treatment.

The subject of the present invention is also a composition having an activity on the proliferation of clonogenic cells by interfering with the generation of clonogenic cells, either by stimulating the proliferation and recruitment, or by inhibiting the proliferation, comprising a therapeutically effective quantity of an isoflavonoid or of an analogous compound of the chromone type, and in particular of a compound chosen from the compounds of formula:



(I)

in which formula:

- R_1, R_2, R_3 and R_4 are chosen, independently of each other, from H, OH, a C_1-C_4 alkoxy group, an $-OCOR_7$ group, R_7 being a C_1-C_4 alkyl group, at least one of the substituents R_1, R_2, R_3 or R_4 being other than H and it being possible for R_2 and R_3 to form together a methylenedioxy group,

- R_5 is chosen from H, OH, a C_1-C_4 alkoxy group, an O-glycosyl group, and a cyclohexyl group,
- R_6 is chosen from a cyclohexyl group, a phenyl group and a phenyl group substituted 1 to 3 times with groups chosen from H, OH and a C_1-C_4 alkoxy group,
- and —— denotes either a double bond, or a single bond.

The subject of the present invention is also the use of an isoflavonoid, in particular of a compound of formula I as defined above, for the manufacture of a medicament intended to interfere (by induction or inhibition) with the generation of clonogenic cells in tumours during a treatment with at least one cytotoxic agent.

In the chemotherapeutic treatment of cancers with cytotoxic agents, the isoflavonoids and in particular the compounds of formula I may be administered at the beginning of the chemotherapy treatments either once, or over several days at the beginning of these treatments (for example for 5 to 7 days) and, depending on the chemotherapy protocol, at the beginning of each treatment cycle (for example for 2 to 5 days) during each cure.

The isoflavonoids and in particular the compounds of formula I are advantageously administered by infusion (generally over 1 to 3 hours) at doses of 5 to 50 mg/kg/day or 200 to 2000 mg/m^2/day.

In order to obtain a maximum effect on the production of clonogenic cells, the isoflavonoids should be administered such that the tissue concentrations obtained are the highest which can be possibly envisaged.

For the treatment protocols in the acute phases of the cures, the intravenous route is to be preferred using:

- ready-to-use infusion solutions (bags, vials and the like) intended to be administered as they are by intravenous infusion with the aid of an infusion line and using the recommended flow rate:

- lyophilizates to be resuspended in solution for intravenous infusion with the aid of pharmaceutical solutions known to persons skilled in the art;

- for the maintenance treatments, it is also possible to envisage the oral route when the chemotherapy treatment preferably uses the administration of cytostatic agents by the oral route. For this purpose, oral lyophilizates (for oral or *perlingual* absorption), instant or delayed release tablets, oral solutions, suspensions, granules, gelatine capsules and the like may be used.

The compounds of formula (I) are, for the majority, compounds of natural origin or are derivatives of compounds of natural origin. As examples, there may be mentioned:

- genistein,
- biochanin A,
- daidzein,
- formononetin,
- 7-acetylformononetin,
- glycetein,
- orobol or 5,7,3',4'-tetrahydroxyisoflavone,
- irizolone or 6,7-methylenedioxy-4'-hydroxyisoflavone,
- irigenin or 3',5,7-trihydroxy-4',5',6-methoxyisoflavone,
- tectorigenin or 4',5,7-trihydroxy-6-methoxyisoflavone,
- 2-hydroxy-8-methoxy-2,3-dihydroisoflavone,
- 4',7-dihydroxy-5-methoxyisoflavone.

Other isoflavones which can be used are described by Donnelly et al. in Natural Product Reports, 1995, 321, or can be prepared by the methods described in this article.

The cytotoxic agents may be chosen from:

i) intercalating agents, in particular doxorubicin (adriamycin), daunorubicin, epirubicin, idarubicin, zorubicin, aclarubicin,

pirarubicin, acridine, mitoxanthrone, actinomycin D, eptilinium acetate;

ii) alkylating agents chosen from platinum derivatives (cisplatin, carboplatin, oxaliplatin and the like),

iii) a compound chosen from the other groups of alkylating agents:

- cyclophosphamide, ifosfamide, chlormetrin, melphalan, chlorambucil, estramustine,
- busulfan, mitomycin C,
- nitrosoureas: BCNU (carmustine), CCNU (lomustine), fotemustine, streptozotocin,
- triazines or derivatives, procarbazine, dacarbazine,
- pipobroman,
- ethyleneimines: altretamine, triethylene-thiophosphoramide,

iv) a compound chosen from the other groups of antimetabolic agents:

- antifolic agents: methotrexate, raltitrexed,
- antipyrimidines: 5-fluorouracil (5-FU), cytarabine (Ara-C),
- hydroxyurea
- antipurines: purinethol, thioguanine, pentostatin, cladribine
- inductors of the synthesis of cytotoxic nucleosides: gemcitabine,

v) a compound chosen from the other groups of agents with high affinity for the tubules:

- vinca alkaloids which disorganize the mitotic spindle: vincristine, vinblastine, vindesine, navelbine
- agents blocking the depolymerization of the mitotic spindle: paclitaxel, docetaxel
- agents inducing breaks in the DNA by inhibition of topoisomerase II: etoposide, teniposide
- inhibitors of topoisomerase I inducing breaks in DNA: topotecan, irinotecan,

vi) an agent breaking, fragmenting DNA, such as bleomycin,

vii) one of the following compounds: plicamycin, L asparaginase, mitoguazone, dacarbazine,

viii) an anticancer progestogenic steroid: medroxyprogesterone, megestrol,

ix) an anticancer oestrogenic steroid: diethylstilbestrol; tetrasodium fosfestrol,

x) an antioestrogen: tamoxifen, droloxifen, raloxifen, aminogluthetimide,

xi) a steroidal antiandrogen (e.g. cyproterone) or a nonsteroidal antiandrogen (flutamide, nilutamide).

In particular, the compounds of formula I may be combined with all the treatments with the major cytotoxic agents used in polychemotherapy of solid tumours such as:

- doxorubicin
- alkylating agents: oxazophorines (cyclophosphamide, ifosfamide, chlorambucil, melphalan)
- nitrosoureas
- mitomycin C
- antimetabolites such as methotrexate, 5-FU, Ara-C, capecitabine
- agents which interfere with tubulin: vinca alkaloids (vincristine, vinblastine, vindesine, navelbine), taxoids (paclitaxel, docetaxel), derivatives of epipodophyllotoxins (etoposide, teniposide)
- bleomycin
- inhibitors of topoisomerase I: topotecan, irinotecan.

Likewise, the compounds of formula I may be combined with the treatment with the major cytotoxic agents used in oncohaematology for the treatment of blood cancers:

- Hodgkin's disease: cyclophosphamide, mechlorethamine, chlorambucil, melphalan, ifosfamide, etoposide, doxorubicin, daunorubicin;

- acute leukaemias: methotrexate, 6-mercaptopurine, cytarabine, vinblastine, vincristine, doxorubicin, daunorubicin, L-asparaginase;

- non-Hodgkin's malignant lymphomas, mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide, methotrexate, cytarabine, vinblastine, vincristine, etoposide, doxorubicin, daunorubicin, carmustine, lomustine, cisplatin;

- chronic lymphoid leukaemias: mechloretamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide.

Results of pharmacological trials demonstrating the effects obtained will be given below.

1 - Interaction (stimulation or inhibition of proliferation) with the generation of clonogenic cells (clonogenic test)

The test used is that described by Hamburger et al. (Science, 1977; 197, 461-463) and Salmon et al. (New England J. Med., 298, 1321-1327). A cell is considered to be clonogenic if it possesses the capacity to proliferate and to give rise to a cell colony. The "human tumour stem cells" are the cells which are at the origin of the neoplastic cells which constitute a given tumour. These tumour stem cells are responsible for the recidivation processes which can be observed after surgical resection of the primary tumours and are also responsible for the formation of metastases. At the level of a tumour or a tumour cell line, these clonogenic stem cells are distinguishable from the other cells of the tumour or the neoplastic cell line considered, by the fact that they retain their capacity to proliferate in the absence of any solid support.

In this test, the tumour cells are cultured on a semisolid support. Only the cells which do not require a solid support for their growth (that is to say the highly tumorigenic cells called "anchorage-independent cells" by M.I. Dawson et al., Cancer Res. 1995; 55: 4446-4451; also called clonogenic cells with

reference to "clonal growth") are capable of developing on such an agar-based support. Indeed, on such a medium, the normal cells - which grow in "adherent mode" ("anchorage-dependent cells" according to the terminology of M.I. Dawson) - such as for example the fibroblasts, do not survive. Within a tumour cell population, cultured on such a support, it is these clonogenic cells (associated with an unlimited number of cell divisions and whose proliferation is called "anchorage-independent [clonal] growth" by M.I. Dawson) which are capable of growing. The percentage of these clonogenic cells within a tumour or a cell line varies between 0.1% and 0.001%. The nonclonogenic cells (associated with a limited number of cell divisions) do not develop in this test because they require a solid support for their growth which should occur in "adherent mode" ("anchorage-dependent [adherent] growth", according to M.I. Dawson et al., Cancer Res. 1995; 55: 4446-51)".

The influence of compounds of formula (I) on the growth of the cell colonies obtained by culturing, for example, the mammary tumour lines MCF7 and MXT and the colorectal line HT-29 on the semiliquid culture medium called "soft agar" was measured. On such a medium, only the clonogenic cells called "anchorage-independent (clonal) cells" by M.I. Dawson survive and develop. The growth of these cells in such a "nonadherent" mode reflects their degree of tumorigenicity. The inhibition of the growth of the size of a tumour in which a larger number of clonogenic cells have developed then becomes the control for a reinforced cytotoxic activity.

By contrast, this test can also reveal that a compound is capable of inhibiting the generation/proliferation of clonogenic cells, which makes the tumour less capable of developing, and therefore reduces the population of tumour cells.

The tumour cell lines studied are maintained in culture in 25 cm^2 falcon flasks. They are then

trypsinized and the cells well dissociated from each other. The percentage of living cells is determined after staining with trypan blue. A cellular suspension at the concentration $5 \cdot 10^4$ to $15 \cdot 10^4$ cells/ml (depending on the cell type considered) is prepared in a 0.3% agar solution. Next, 200 µl of this suspension are inoculated into Petri dishes 35 mm in diameter, in which 3 ml of a bottom layer consisting of a 0.5% agar solution are deposited. The 200 µl of cellular suspension are in turn covered with 1.8 ml of a top layer consisting of a 0.3% agar solution. The dishes are then placed in an incubator at 37°C, 5% CO_2 and 70% humidity until the treatment. The latter is performed about 1 to 2 hours after inoculation. The compounds to be tested are prepared at a concentration 100-fold greater than the desired concentration and 50 µl of these treating solutions are deposited on the agar top layer of the corresponding dishes. In the present study, the final concentration of the products tested is 10^{-5}, 10^{-7} and 10^{-9} M. The dishes are then maintained in the incubator for 21 days. On the 21st day, the dishes are treated by depositing on the top layer 100 µl of a solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide) at 1 mg/ml prepared with RPMI 1640 medium for 3 h at 37°C. After this period of time, the cell colonies are fixed by adding 2 ml of formalin per dish. After fixing for 24 hours, the formalin is evaporated and a number of coloured cell colonies, therefore consisting of metabolically active cells, and whose surface area is greater than 100 μm^2, is determined with the aid of an inverted microscope.

The average number of clonogenic cell clones determined for each experimental condition studied is expressed as a percentage relative to the average number of clonogenic cell clones counted under the control condition and posed as equal to 100%. These values, expressed as the percentage relative to the control condition, are presented in Table I.

TABLE I

CELL LINES	Genistein (in mol.l^{-1})		
	10^{-5}	10^{-7}	10^{-9}
MCF7	66.9 ± 2.9 **	74.2 ± 4.7 *	89.2 ± 0.9 NS
HT-29	118.2 ± 2.8 **	108.9 ± 2.3 *	104.6 ± 2.5 NS
MXT	71 ± 2.5 **	118.5 ± 2.2 **	117.5 ± 2.2 **

- The results summarized in this table represent the mean values ± standard error of the mean (SEM) established on at least 6 wells.

- Control condition = 100%

- (NS: p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001).

Depending on the cell line studied, genistein can:

- recruit the clonogenic cells inside the tumour (cell lines HT-29 at the concentrations of 10^{-5} M and 10^{-7}, and MXT at the concentrations of 10^{-7} M and 10^{-9} M), that is to say induce a significant increase in the number of colonies of these cells compared with that obtained under the control condition, and then makes them more sensitive to the conventional treatment with cytotoxic agents, or

- be capable of directly inhibiting the proliferation of these clonogenic cells (MCF7 cell line at the concentrations of 10^{-5} M and 10^{-7} M).

2 - Cytotoxic activity at the level of the nonclonogenic cells: "MTT test"

The influence of the compounds of formula (I) on the nonclonogenic cells was evaluated with the aid of the MTT colorimetric test.

The principle of the MTT test is based on the mitochondrial reduction by metabolically active living cells of the product MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which is yellow in

colour, to a product which is blue in colour, formazan. The quantity of formazan thus obtained is directly proportional to the quantity of living cells present in the culture well(s). This quantity of formazan is measured by spectrophotometry.

The cell lines are maintained in monolayer culture at 37°C in closed-stopper culture dishes containing basal medium MEM 25 MM HEPES (Minimum Essential Medium). This medium is quite suitable for the growth of a range of varied mammalian diploid or primary cells. This medium is then supplemented:

- with a quantity of 5% of decomplementized SVF (Foetal Calf Serum) at 56°C over 1 hour,
- with 0.6 mg/ml of L-glutamine,
- with 200 IU/ml of penicillin,
- with 200 µg/ml of streptomycin,
- with 0.1 mg/ml of gentamicin.

The 12 human cancer cell lines which were used were obtained from the *American Type Culture Collection* (ATCC, Rockville, MD, USA). These 12 cell lines are:

- U-373MG (ATCC code: HTB-17) and U-87MG (ATCC code: HTB-14) which are two glioblastomas,
- SW1088 (ATCC code: HTB-12) which is an astrocytoma,
- A549 (ATCC code: CCL-185) and A-427 (ATCC code: HTB-53) which are two non-small-cell lung cancers,
- HCT-15 (ATCC code: CCL-225) and LoVo (ATCC code: CCL-229) which are two colorectal cancers,
- T-47D (ATCC code: HTB-133) and MCF7 (ATCC code: HTB-22) which are two breast cancers,
- J82 (ATCC code: HTB-1) and T24 (ATCC code: HTB-4) which are two cancers of the bladder,
- PC-3 (ATCC code: CRL-1435) which is a prostate cancer.

From the experimental point of view, 100 µl of a cellular suspension containing 20,000 to 50,000 (according to the cell type used) cells/ml of culture

medium are inoculated into flat-bottomed 96-well multi-well plates and are incubated at 37°C, under an atmosphere comprising 5% CO_2 and 70% humidity. After 24 hours of incubation, the culture medium is replaced with 100 µl of fresh medium containing either the various compounds to be tested at concentrations varying from 10^{-5} to 10^{-10} M, or the solvent which served for the dissolution of the products to be tested (control condition). After 72 hours of incubation under the preceding conditions, the culture medium is replaced with 100 µl of a yellowish solution of MTT dissolved in an amount of 1 mg/ml in RPMI 1640. The microplates are incubated for 3 hours at 37°C and then centrifuged for 10 minutes at 400 g. The yellowish solution of MTT is removed and the blue formazan crystals formed in the cell are dissolved in 100 µl of DMSO. The microplates are then placed under stirring for 5 minutes. The intensity of the resulting blue colour, and therefore of the conversion of the yellow MTT product to blue formazan by the cells still alive at the end of the experiment, is quantified by spectrophotometry with the aid of a DYNATECH IMMUNOASSAY SYSTEM type apparatus at the wavelengths of 570 nm and 630 nm corresponding to the wavelengths for maximum absorption of formazan and to the background noise, respectively. A software integrated into the spectrophotometer calculates the mean optical density values as well as the standard deviation (Std. Dev.) and standard error of the mean (SEM) values.

By way of nonlimiting example, the results of the mean optical density, expressed as a percentage relative to the mean optical density measured under the control condition (posed equal to 100%), obtained with an isoflavonoid: genistein, on the 5 tumour cell lines U-87MG, J82, HCT-15, T-47D and A549, will be given in Table II.

TABLE II

CELL LINES	Genistein (in mol.l^{-1})					
	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
U-87MG	83.8 ± 3.5 **	98.1 ± 4.4 NS	94.3 ± 3.7 NS	100.1 ± 6.6 NS	98.2 ± 3.5 NS	108.6 ± 2.3 *
J82	87.0 ± 1.0 ***	99.3 ± 1.1 NS	101.6 ± 0.8 NS	101.8 ± 1.8 NS	102.8 ± &.5[sic] NS	104.2 ± 1.5 NS
HCT-15	96.8 ± 5.3 NS	100.9 ± 6.0 NS	97.5 ± 5.2 NS	89.2 ± 3.5 *	89.4 ± 4.0 *	90.5 ± 3.3 *
T-47D	92.3 ± 2.2 *	98.9 ± 3.3 NS	95.1 ± 1.6 NS	97.8 ± 3.0 NS	100.0 ± 3.4 NS	102.4 ± 1.7 NS
A-549	81.4 ± 4.8 **	105.0 ± 4.1 NS	101.6 ± 5.4 NS	106.0 ± 3.2 NS	108.9 ± 2.1 *	103.6 ± 3.9 NS

- xx ± yy = mean value ± standard error of the mean
- control condition = 100%
- (NS: p >0.05; *: p <0.005; **; p <0.01; ***: p < 0.001).

Genistein has a low antitumour power. This nontoxic product induces, when it is the case, inhibition of the overall cell proliferation of these lines only at the concentration of 10^{-5} M and this inhibition does not exceed 20%. At the other concentrations tested, only a few marginal effects can be demonstrated.

3. - Determination of the maximum tolerated dose (MTD):

The evaluation of the maximum tolerated dose was carried out in 4- to 6-week old B6D2F1/Jico mice. The compounds were administered by the intraperitoneal route in increasing doses ranging from 2.5 to

160 mg/kg. The value of the MTD (expressed in mg/kg) is determined from the observation of the rate of survival of the animals over a period of 14 days after a single administration of the product considered. The variation of the weight of the animals is also monitored over this period. When the MTD value is greater than 160 mg/kg, the MTD value is considered to be 160 mg/kg by default.

Genistein is by default associated with an MTD equal to 160 mg/kg. This result suggests that the products of the isoflavonoid family do not exhibit any direct toxicity and can be used in high tissue concentrations, and therefore in high dosages.

4. - Antitumour activity in vivo in combination with a cytotoxic agent

The trials were carried out on the models of:

- hormone-sensitive murine mammary adenocarcinoma MXT (HS-MXT),
- lymphoma P 388,

in the presence or otherwise of cytotoxic agents such as cyclophosphamide, etoposide, doxorubicin or vincristine.

When the MTD value for a product was determined, its *in vivo* antitumour activity was characterized at the doses of MTD/2, MTD/4 and MTD/8 on the model of mammary adenocarcinoma of murine origin HS-MXT and on the lymphoma P388 model). It is the dose which exhibited the best antitumour activity on these different models which was selected and used in the context of the treatments combined with the cytotoxic agents.

In all the examples presented below, whatever the model (mammary adenocarcinoma HS-MXT or lymphoma P 388), the control condition is represented by a group of 9 mice to which a volume of 0.2 ml of physiological saline containing the solvent used to dissolve the different compounds of formula (I) used is administered for 5 consecutive weeks and at the rate of

5 administrations (Monday, Tuesday, Wednesday, Thursday and Friday) per week.

The following were determined during these trials:

i) - <u>rate of survival of the mice</u>

This rate of survival was calculated in the form of a ratio T/C:

$$T = \frac{\text{(Number of days of survival of the median mouse of the treated mouse group)}}{\text{(Treated median mouse)}} - \frac{\text{(Number of mice which died during the days which preceded that for the treated median mouse)}}{\text{(Number of mice which died on the same day as the treated median mouse)}}$$

$$C = \frac{\text{(Number of days of survival of the median mouse of the treated mouse group)}}{\text{(Treated median mouse)}} - \frac{\text{(Number of mice which died during the days which preceded that for the control median mouse)}}{\text{(Number of mice which died on the same day as the control median mouse)}}$$

This ratio represents the mean survival time for the median mouse of the treated mouse group relative to the mean survival time for the median mouse of the control mouse group. Thus, a molecule induces a significant increase ($P < 0.05$) in the survival of the animals when the T/C ratio exceeds 130%. On the other hand, it exhibits a toxic effect when this T/C value is less than 70%.

ii) - <u>tumour growth</u> by measuring, twice per week (Monday and Friday), the surface area of the transplanted HS-MXT and P388 tumours. This surface area

is calculated by taking the product of the value of the two largest perpendicular axes of the tumour. The value of these axes is measured with the aid of a slide calliper.

4.1. Murine mammary adenocarcinoma (HS-MXT)

The model of murine mammary adenocarcinoma MXT which is hormone-sensitive (HS-MXT) transplanted in 4- to 6-week old B6D2F1/Jico mice is a model derived from the galactophorous ducts of the mammary gland (Watson C. *et al*. Cancer Res. 1977; 37: 3344-48).

The results obtained using genistein either alone or in combination with the cytotoxic agents will be given by way of example.

Treatment 1

Genistein is administered alone. The first injection of the product is carried out on the seventh day post-transplantation (D7) for four consecutive weeks at the rate of 5 injections per week (Monday, Tuesday, Wednesday, Thursday and Friday) and at the dose of 20 mg/kg.

Treatment 2

Cyclophosphamide is administered alone. The first injection of the product is carried out on the fourteenth day post-transplantation (D14) for three consecutive weeks at the rate of 3 injections per week (Monday, Wednesday, and Friday) and at the dose of 10 mg/kg.

Treatment 3

Vincristine (VCR) is administered alone. The first injection of the product is carried out on the fourteenth day post-transplantation (D14) for three consecutive weeks at the rate of 3 injections per week (Monday, Wednesday, and Friday) and at the dose of 0.63 mg/kg.

Treatment 4

Etoposide (ETO) is administered alone. The first injection of the product is carried out on the

fourteenth day post-transplantation (D14) for three consecutive weeks at the rate of 3 injections per week (Monday, Wednesday, and Friday) and at the dose of 10 mg/kg.

Treatment 5

Genistein is coadministered with cyclophosphamide. In this case, the first injection of genistein is carried out on the seventh day post-transplantation (D7) for four consecutive weeks at the rate of 5 injections per week (Monday, Tuesday, Wednesday, Thursday and Friday) at the dose of 20 mg/kg and the first injection of cyclophosphamide is carried out on the fourteenth day post-transplantation (D14) for three consecutive weeks at the rate of three injections per week (Monday, Wednesday and Friday) at the dose of 10 mg/kg.

Treatment 6

Genistein is coadministered with vincristine. In this case, the first injection of genistein is carried out on the seventh day post-transplantation (D7) for four consecutive weeks at the rate of 5 injections per week (Monday, Tuesday, Wednesday, Thursday and Friday) at the dose of 20 mg/kg and the first injection of vincristine is carried out on the fourteenth day post-transplantation (D14) for three consecutive weeks at the rate of three injections per week (Monday, Wednesday and Friday) at the dose of 0.63 mg/kg.

Treatment 7

Genistein is coadministered with etoposide. In this case, the first injection of genistein is carried out on the seventh day post-transplantation (D7) for four consecutive weeks at the rate of 5 injections per week (Monday, Tuesday, Wednesday, Thursday and Friday) at the dose of 20 mg/kg and the first injection of etoposide is carried out on the fourteenth day post-transplantation (D14) for three consecutive weeks at the rate of three injections per week (Monday, Wednesday and Friday) at the dose of 10 mg/kg.

The results obtained for the survival period (Table III) for genistein will be given below.

TABLE III

Treatments	T/C (expressed in %)
1 (genistein)	100
2 (CPA)	107
3 (VCR)	105
4 (ETO)	116
5 (genistein + CPA)	131
6 (genistein + VCR)	135
7 (genistein + ETO)	131

These results show that the coadministration of genistein with the cytotoxic agents: cyclophosphamide, vincristine or etoposide, significantly increases the mean survival time for the median mouse of the different groups of mice thus treated compared with the mean survival time for the median mouse of the control mouse group. Furthermore, this increase in the mean survival time for the median mouse of the different groups of mice treated with these coadministrations is significantly longer than that obtained with the treatments involving genistein or these cytotoxic agents used alone.

The study of tumour growth moreover showed the following results. In Table IV below are indicated, in per cent, the decreases (-) or the increases (+) in the surface area of the HS-MXT tumours induced with the different treatments 1, 2, 3, 4, 5, 6 and 7 compared with the control condition on the 28th day after the tumour transplantation, that is after 15 administrations of genistein and 6 administrations of the different cytotoxic agents used or otherwise in coadministrations with genistein. On the 28th day post-transplantation, 89% of the control animals are still alive (that is 8 animals out of 9).

Table IV

Treatments	Variation in the tumour surface area (expressed in %)
1 (genistein)	+ 2.6
2 (CPA)	- 25
3 (VCR)	- 32
4 (ETO)	- 22
5 (genistein + CPA)	- 20
6 (genistein + VCR)	- 45
7 (genistein + ETO)	- 41

These results show that the coadministration of genistein with the cytotoxic agents: vincristine and etoposide, significantly induces a decrease in the growth of the HS-MXT tumours which is greater than that induced by the treatments involving genistein alone (which has no relevant clinical effect) or the latter two cytotoxic agents used alone.

4.2. Lymphoma P 388:

The 4- to 6-week old CDF1 mice receive a transplant consisting of a piece of P388 tumour (obtained from a bank of tumours maintained in the laboratory) subcutaneously on the right side on day D0. In order to be in a situation similar to the clinical reality, we wait for the 5th day post-transplantation (D5) before starting the treatment. This was because, after this period of time, the subcutaneous P388 tumours are palpable.

By way of example, the results obtained with genistein alone or in combination with vincristine are reported below.

Treatment 1

Genistein is administered alone. The first injection of the product is carried out on the fifth day post-transplantation (D5) at the rate of 5 injections per week (Monday, Tuesday, Wednesday,

Thursday and Friday) for five consecutive weeks and at the dose of 40 mg/kg.

Treatment 2

Vincristine (VCR) is administered alone. The first injection of the product is carried out on the fifth day post-transplantation (D5) at the rate of 3 injections per week (Monday, Wednesday and Friday) for three consecutive weeks and at the dose of 0.63 mg/kg.

Treatment 3

Genistein is coadministered with vincristine. In this case, the first injection of genistein is carried out on the fifth day post-transplantation (D5) at the rate of 5 injections per week (Monday, Tuesday, Wednesday, Thursday and Friday) for five consecutive weeks at the dose of 40 mg/kg and the first injection of vincristine is carried out on the fifth day post-transplantation (D5) at the rate of 3 injections per week (Monday, Wednesday and Friday) for three consecutive weeks at the dose of 0.63 mg/kg.

The results obtained with treatments 1, 2 and 3 on the survival times for the mice are presented below in Table 5.

Table V

Treatments	T/C (expressed in %)
1 (genistein)	125
2 (VCR)	122
3 (genistein + VCR)	169

These results show that the coadministration of genistein with vincristine increases in a very highly significant manner the mean survival time for the median mouse of the different groups of mice thus treated compared with the mean survival time for the median mouse of the control mouse group. Furthermore, this increase in the mean survival time for the median mouse of the different groups of mice thus treated is highly significant compared with the mean survival time

for the median mouse of the different groups of mice treated with genistein or vincristine which are used alone.

Examples of the modality of using the compounds of formula I in mono- or polychemotherapy protocols with cytotoxic agents will be given below.

A. Solid tumours

1/ Lung cancers

1.1. Non-small-cell type (advanced stage):

- to the recommended protocol (T. Le Chevalier et al., J. Clin. Oncol. 1994; 12. 360-367), the intravenous infusions of genistein or of another isoflavonoid are added:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1, D_8, D_{15}, D_{22}, D_{29} and D_{36}
• navelbine	30 mg/m²/day	i.v.	D_1, D_8, D_{15}, D_{22}, D_{29} and D_{36}
• cisplatin	120 mg/m²	i.v.	D_1 and D_{29}

this cure is repeated 8 times.

1.2. Small-cell type (advanced stage):

- to the recommended CAV or VAC protocol (B.J. Roth et al., J. Clin. Oncol. 1992; 10: 282-291), the isoflavonoid infusions are added:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1
• cyclophophamide	1000 mg/m² bolus	i.v.	D_1
• doxorubicin	40 to 50 mg/m² bolus	i.v.	D_1
• vincristine	1 to 1.4 mg/m² bolus (max 2 mg)	i.v.	D_1

this cure is to be repeated 6 times every 21 days.

- to the recommended Pt-E protocol (B.J. Roth et al., J. Clin. Oncol. 1992; 10: 282-291) the genistein infusions are added

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• cisplatin	20 mg/m²/day infusion of 20 to 60 minutes	i.v.	D_1-D_5
• etoposide	80 mg/m²/day infusion of 60 minutes	i.v.	D_1 - D_5

each cycle is repeated every 21 days and the cure comprises 6 cycles.

1.3. Non-small-cell bronchial cancer, locally advanced or metastatic:

- monochemotherapy:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1, D_8, D_{15} then 1 week/rest
• gemcitabine	1000 mg/m²/day infusion of 0.5 hour	i.v.	D_1, D_8, D_{15} then 1 week/rest

it being possible for the cure to comprise the repetition of the cycle of 4 weeks.

- gemcitabine/cisplatin combination:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{15}

• gemcitabine	1000 mg/m²/day infusion of 0.5 hour	i.v.	D_1, D_8, D_{15}
• cisplatin	20 mg/m²/day infusion of 20-60 minutes	i.v.	D_1

the cure comprising the repetition of this cycle every 21 days.

2/ Breast cancers

- CMF protocol as adjuvant treatment for operable breast cancer (G. Bonnadonna et al., N. Engl. J. Med.; 1976; 294: 405-410):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1 to D_{14}
• cyclophosphamide	100 mg/m²/day	oral	D_1 to D_{14}
• methotrexate	40 mg/m² bolus	i.v.	D_1 and D_8
• 5-FU	600 mg/m²	i.v.	D_1 and D_8

each cycle is repeated every 28 days and the cure comprises 6 cycles.

- AC protocol (B. Fisher et al., J. Clin. Oncol.; 1990; 8: 1483 - 1496) as adjuvant treatment:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1
• doxorubicin	60 mg/m² bolus	i.v.	D_1
• cyclophosphamide	600 mg/m² bolus	i.v.	D_1

each cycle is repeated every 21 days and the cure comprises 4 cycles.

- Breast cancers with metastases:

- in the FAC protocol (A.U. Buzdar et al., Cancer 1981; 47: 2537-2542) and its different adaptations, the isoflavonoid infusions are added according to the following scheme (nonlimiting):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m^2/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 and D_8-D_{12} or D_1-D_5
• 5-FU	500 mg/m^2/day bolus	i.v.	D_1 and D_8 or D_1-D_2
• doxorubicin	50 mg/m^2 bolus	i.v.	D_1 or D_1 and D_2
• cyclophos-phamide	500 mg/m^2	bolus i.v. or oral	D_1 D_1

each cycle is repeated every 3 weeks until a new progression of the disease is diagnosed.

- in the CAF protocol (G. Falkson et al., Cancer 1985; 56: 219-224):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m^2/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_{14}
• cyclophos-phamide	100 mg/m^2/day	oral	D_1-D_{14}
• doxorubicin	30 mg/m^2	i.v.	D_1 and D_8
• 5-FU	500 mg/m^2 bolus	i.v.	D_1 and D_8

each cycle is repeated every 28 days until a new progression of the disease is diagnosed.

- in the CMF protocol:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 and D_8-D_{12}
• cyclophos-phamide	600 mg/m²/day bolus	i.v.	D_1 and D_8
• methotrexate	40 mg/m²/day bolus	i.v.	D_1 and D_8
• 5-FU	600 mg/m²/day bolus	i.v.	D_1 and D_8

this cycle is to be repeated every 3 to 5 weeks and the cure comprises 6 cycles.

- in the CMF-VP protocol:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 D_8-D_{12} D_{15}-D_{19} D_{22}-D_{26}
• cyclophos-phamide	2 to 2.5 mg/kg/day	oral	daily
• methotrexate	25 to 50 mg/m²/day	i.v.	D_1, D_8, D_{15}, D_{22}
• 5-FU	300 to 500 mg/m²/day	i.v.	D_1, D_8, D_{15}, D_{22}
• vincristine	0.6 to 1.2 mg/m²/day	i.v.	D_1, D_8, D_{15}, D_{22}
• prednisone	30 mg/m²/day	oral	from D_1 to D_{10}

this cure is to be repeated every 4 weeks.

- in the FEC protocol:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 and D_8-D_{12}
• 5-FU	600 mg/m²/day	i.v.	D_1 and D_8
• epirubicin	50 mg/m²	i.v.	D_1
• cyclophos-phamide	600 mg/m²	i.v.	D_1

this cure is to be repeated every 3 weeks.

- in the MMC-VBC protocol (C. Brambilla et al., Tumori, 1989; 75: 141-144):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 and D_{15}-D_{19}
• mitomycin C	10 mg/m² bolus	i.v.	D_1
• vinblastine	50 mg/m²/day bolus	i.v.	D_1 and D_{15}

this cure is to be repeated every 28 days until progression of the disease is diagnosed.

- in the NFL protocol (S.E. Jones et al., J. Clin. Oncol. 1991; 9: 1736 - 1739):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• mitoxantrone	10 mg/m² bolus	i.v.	D_1
• 5-FU	1000 mg/m² as an infusion of 24 hours	i.v.	D_1-D_3
• leucovorin	100 mg/m² bolus	i.v.	D_1

the cure comprises two cycles 21 days apart and then requires evaluation.

The isoflavonoid infusions may also be combined with the treatment of breast cancers with metastases when a taxoid is used, for example:

- with paclitaxel (F.A. Holmes et al., J. Natl Cancer Inst. 1991; 83: 1797 - 1805) in the treatment of the forms with metastases which may be resistant to anthracyclines:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• paclitaxel	175 mg/m² as an infusion of 3 to 24 hours	i.v.	D_1

This cycle is repeated every 21 days until a new progression of the disease is diagnosed.

- with docetaxel (C.A. Hudis et al., J. Clin. Oncol. 1996; 14: 58-65), in locally advanced or metastatic breast cancer, resistant or in relapse after cytotoxic chemotherapy (which comprised an anthracycline) or in relapse during an adjuvant treatment:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• docetaxel	100 mg/m² or 60-100 mg/m² as an infusion of 1 hour (or of 24 hours)	i.v.	D_1

This cycle is repeated every 21 days for a cure of 2 cycles or until a progression of the disease appears.

- in the dose intensification protocols combining a transplantation of autologous medullary cells and peripheral blood stem cells as a consolidation of the first line treatment, for example:
- CPB protocol (W.P. Peters et al., J. Clin. Oncol. 1993; 11: 132 - 1143), in which the i.v. infusion of stem cells takes place on days D_{-1}, D_0 and D_1:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_{-6} to D_{-1}
• cyclophosphamide	1875 mg/m² as an infusion of 1 hour	i.v.	D_{-6} to D_{-4}
• cisplatin	55 mg/m²/day as a continuous infusion of 24 hours	i.v.	D_{-6} to D_{-4}
• carmustine (BCNU)	600 mg/m²/day as an infusion of 2 hours	i.v.	D_{-3}

- CTCb protocol (K. Antman et al., J. Clin. Oncol. 1992; 10: 102-110), in which the i.v. infusion of stem cells takes place on day D_0:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_{-7} to D_{-1}
• cyclophosphamide	1500 mg/m² as a continuous infusion of 24 hours (4 doses)	i.v.	D_{-7} to D_{-3}
• thiotepa	125 mg/m² as a continuous infusion of 24 hours (4 doses)	i.v.	D_{-7} to D_{-3}
• carboplatin	200 mg/m² as a continuous infusion of 24 hours (4 doses)	i.v.	D_{-7} to D_{-3}

- CTM protocol (L.E. Damon et al., J. Clin. Oncol. 1989; 7: 560-571 and I.C. Henderson et al., J. Cellular Biochem. 1994 (Suppl 18B): 95) in which the i.v. infusion of haematopoietic stem cells takes place on D_0:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_{-6} to D_{-1}
• cyclophosphamide	1500 mg/m²/day as an infusion of 1 hour	i.v.	D_{-6} to D_{-3}
• thiotepa	150 mg/m²/day as an infusion of 2 hours	i.v.	D_{-6} to D_{-3}
• mitoxantrone	10 - 15 mg/m² as an infusion of 1 hour	i.v.	D_{-6} to D_{-3}

3/ **Gynaecological cancers**

3.1. Ovarian cancer:

- for the treatment of in particular metastatic ovarian carcinomas:

i) **PAC protocol** (G.A. Omura et al. J. Clin. Oncol. 1989; 7: 457 - 465): the infusions of isoflavonoids are administered according to the following scheme:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• cisplatin	50 mg/m² (or 40-90 mg/m²) infusion of 1 to 2 hours	i.v.	D_1
• doxorubicin	50 mg/m² bolus (or 30 to 50 mg/m²)	i.v.	D_1
• cyclophosphamide	1000 mg/m² infusion of 1 to 2 hours (or 200 to 600 mg/m²)	i.v.	D_1

this cycle is repeated every 21 to 28 days and the cure comprises 8 cycles.

ii) altretamine protocol, according to A. Marietta et al. (Gynecol. Oncol. 1990; 36: 93-96):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 D_8-D_{12}
• altretamine	200 mg/m²/day divided into 4 doses	oral	D_1-D_{15}

the cure comprising two cycles, 28 days apart.

ii) paclitaxel protocol: the isoflavonoids may be added to the paclitaxel protocol as has been described by W.P. McGuire et al. (Ann. Intern. Med. 1989; 111: 273-279):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3
• paclitaxel	135 mg/m² infusion of 3 hours or of 24 hours	i.v.	D_1

the cure comprising two of these cycles, 28 days apart (with evaluation at the end).

- for the treatment of metastatic and refractory ovarian carcinomas, the isoflavonoids may be added to the second line protocol, based on topotecan:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• topotecan	1.5 mg/m²/day infusion of 0.5 hour	i.v.	D_1-D_5

the cure comprising two cycles, 21 days apart (with evaluation at the end)

according to A.P. Kudelka et al. (J. Clin. Oncol. 1996: 14: 1552-1557).

3.2 Trophoblastic tumours:

- in low-risk patients, the isoflavonoids may be combined with the protocol described by H. Takamizawa et al. (Semin. Surg. Oncol. 1987; 3: 36 - 44):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• methotrexate (MTX)	20 mg/day	i.m.	D_1-D_5
• dactinomycin (DACT)	0.5 mg/day as a bolus	i.v.	D_1-D_5

(MTX-DATC protocol).

3.3 Uterine cancers:

- the isoflavonoids may also be combined with the CAV (or VAC) protocol according to the scheme below:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3
• cyclophosphamide	750 - 1200 mg/m² as an infusion	i.v.	D_1
• doxorubicin	45-50 mg/m² as an infusion	i.v.	D_1
• vincristine	1.4 mg/m²	i.v.	D_1

the cure comprising a repetition of this cycle every 21 days.

- in the FAP protocol:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• fluorouracil (5-FU)	600 mg/m²/day	i.v.	D_1, D_8
• doxorubicin	30 mg/m³	i.v.	D_1
• cisplatin	75 mg/m²	i.v.	D_1

the cure comprising the repetition of this cycle every 21 or 28 days.

4/ Testicular and prostate cancers

- the isoflavonoids may also be combined with the testicular cancer protocols:

BEP protocol:	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• bleomycin	30 mg/m² as an infusion	i.v.	D_1
• etoposide	100 mg/m²/day as an infusion	i.v.	D_1-D_5
• cisplatin	20 mg/m²/day	i.v.	D_1-D_5

the cure comprising three cycles, at the rate of one cycle every 21 days.

5/ Bladder cancers

- the isoflavonoids may be combined with the CISCA2 (also called PAC) protocol

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• cisplatin	50 mg/m²	i.v.	D_1
• cyclophosphamide	600 mg/m³ as an infusion	i.v.	D_1
• doxorubicin	75 mg/m²	i.v.	D_1

	as an infusion		

the cycle having to be repeated every 3 weeks.

- in the MVAC protocol (according to CN Sternberg et al., J. Urol. 1988; 139: 461-469):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3 D_{15}-D_{18} D_{22}-D_{25}
• methotrexate	30 mg/m² bolus	i.v.	D_1, D_{15}, D_{22}
• vinblastine	3 mg/m²	i.v.	D_2 or D_2, D_{15}, D_{22}
• doxorubicin	30 mg/m² bolus	i.v.	D_2
• cisplatin	70-100 mg/m² infusion of 1 h	i.v.	D_1 or D_2

this cycle being repeated every 4 to 5 weeks, at least for 2 cycles.

6/ Nasopharyngeal carcinomas/head and neck cancers

- The isoflavonoids may be legitimately combined with the polychemotherapy protocols used in the treatment of these cancers:

6.1 Nasopharyngeal cancers:

- ABVD protocol:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3 D_8-D_{10} or D_{15}-D_{17}
• doxorubicin	30 mg/m²/day	i.v.	D_1 and D_8 or D_{15}
• bleomycin	10 mg/m²/day	i.v.	D_1 and D_8 or D_{15}
• vinblastine	6 mg/m²/day	i.v.	D_1 and D_8 or D_{15}
• dacarbazine	200 mg/m²/day	i.v.	D_1 and D_8 or D_{15}

the cure comprising 1 to 6 cycles repeated at the rate of 1 cycle every 4 weeks.

6.2 Head and neck cancers with metastases:

- in the Pt-FU protocol (e.g.: for cancers of the pharynx): according to the DVAL Study Group (New Engl. J.M. 1991; 324: 1685 - 1690):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• cisplatin	100 mg/m² infusion of 1 h	i.v.	D_1
• fluorouracil (5-FU)	1000 mg/m²/day continuous infusion	i.v.	D_1-D_5

the cure comprising two cycles, at the rate of 1 cycle every 3 weeks.

7/ Carcinomas of the soft tissues

- The isoflavonoids may be introduced in a protocol such as the CYVADIC protocol:
- according to H.M. Pinedo et al. (Cancer 1984; 53: 1825):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3 D_8-D_{10} D_{15}-D_{17}
• cyclophosphamide (Cy)	500 mg/m² bolus	i.v.	D_2
• vincristine (V)	1.5 mg/m²/day bolus	i.v.	D_1, D_8, D_{15},
• doxorubicin (A)	50 mg/m² bolus	i.v.	D_2
• dacarbazine (DIC)	250 mg/m²/day infusion of 15 minutes	i.v.	D_1-D_5

the cure comprising the repetition of this cycle every 4 weeks, first for 2 cycles.

8/ **Hormone-refractory prostate cancer, with metastases**

- in the VBL-estramustine, according to G.R. Hudis et al. (J. Clin. Oncol. 1992; 10: 1754:1761):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3, D_8-D_{10} D_{15}-D_{17}. D_{22}-D_{24}, D_{29}-D_{31}, D_{36}-D_{38}
• vinblastine	4 mg/m²/day bolus	i.v.	D_1, D_8, D_{15}, D_{22}, D_{29}, D_{36}
• estramustine	200 mg/m²/day tid (600 mg/m²/day)	oral	every day for 6 weeks

a treatment cycle lasting for 6 weeks and being followed by 2 weeks of free interval.

9/ **Cancers of the germ cells**

i) for tumours with a favourable prognosis:

- Pt-E protocol, according to G.J. Bosl et al. (J. Clin. Oncol. 1988: 6: 1231-1238)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• cisplatin (Pt)	20 mg/m²/day infusion of 20 to 60 minutes	i.v.	D_1-D_5
• etoposide (E)	100 mg/m²/day infusion of 1 hour	i.v.	D_1-D_5

the cure comprising 4 cycles, at the rate of 1 cycle every 21 or 28 days.

ii) for tumours with metastases:

- PEB protocol, according to S.D. Williams et al. (N. Eng. J. Med. 1987; 316: 1435-1440):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m^2/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 D_9-D_{11} D_{16}-D_{18}
• cisplatin (P)	20 mg/m^2/day infusion of 20 to 1 h	i.v.	D_1-D_5
• etoposide (E)	100 mg/m^2/day infusion of 1 h	i.v.	D_2, D_9, D_{16}
• bleomycin (B)	30U (or mg)/day bolus	i.v.	D_1-D_5

the cure comprising 4 cycles, at the rate of 1 cycle every 21 days.

10/ <u>Kidney cancers</u>

- **metastatic renal carcinoma:** the isoflavonoids may be introduced in the protocol described by M.J. Wilkinson et al. (Cancer 1993; 71: 3601-3604):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m^2/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 D_8-D_{15}
• floxuridine	0.075 mg/kg/day continuous infusion	i.v.	D_1-D_{14}

the cure comprising two cycles 28 days apart.

- **nephroblastoma:** the isoflavonoids may be introduced in the DAVE protocol:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3 D_8-D_{10}
• dactinomycin	0.6 mg/m²/day	i.v.	D_1, D_8
• doxorubicin	30 mg/m²/day	i.v.	D_1, D_8
• cyclophosphamide	200 mg/m²/day infusion of 1 hour	i.v.	D_1, D_8

at the rate of one cycle every 3 to 4 weeks.

11/ Cancers of the digestive tube

11.1 Cancers of the oesophagus:

- the isoflavonoids may be introduced in the FAP protocol according to:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3 D_8-D_{10}
• 5-fluorouracil (5-FU)	600 mg/m²	i.v.	D_1, D_8
• doxorubicin	30 mg/m²	i.v.	D_1
• cisplatin	75 mg/m²	i.v.	D_1

this cycle being repeated every 3 to 4 weeks.

11.2 Stomach cancers

- in advanced gastric carcinomas and/or with metastases:
- EAP protocol (according to P. Preusser et al., J. Clin. Oncol. 1989; 7: 1310):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{10}
• etoposide	120 mg/m²/day infusion of 1 hour	i.v.	D_3, D_4, D_5 or D_4-D_6
• doxorubicin	20 mg/m²/day bolus	i.v.	D_1, D_7

• cisplatin	40 mg/m²/day infusion of 1 hour	i.v.	D_2, D_8

at the rate of 1 cycle every 28 days.

- FAMtx protocol: according to J.A. Wils et al. (J. Clin. Oncol. 1991; 89; 827):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3
• fluorouracil (5-FU) (F)	1500 mg/m² bolus 1 hour after methotrexate	i.v.	D_1
• doxorubicin (A)	30 mg/m² bolus	i.v.	D_{15}
• methotrexate (Mtx)	1500 mg/m² infusion of 30 minutes	i.v.	D_1

the cure first comprising two cycles, 28 days apart.

- in certain patients, the protocol or its variant (epirubicin replacing doxorubicin) may be used according to the following scheme:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3
• fluorouracil (5-FU)	1500 mg/m²	i.v.	D_1
• doxorubicin (A)	30 mg/m² bolus	i.v.	D_1 = $FAMT_x$
<u>or</u>			
• epirubicin (A)	60 mg/m² bolus	i.v.	D_1 = $FEMT_x$
• methotrexate (to be infused before 5-FU)	1500 mg/m²	i.v.	D_1
• leucovorin	15 mg/m²/day	oral	D_2-D_4

12/ **<u>Colorectal cancers</u>**

- the isoflavonoids may be introduced in the protocol for FU-Levamisole adjuvant treatment

of colorectal cancer (according to C.G. Moertel et al., N. Eng. J. Med. 1990; 322: 352):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 D_{29}-D_{31}
• 5-fluorouracil	450 mg/m²/day bolus	i.v.	D_1-D_5
• 5-fluorouracil	450 mg/m² bolus	i.v.	D_{29}
• levamisole	50 mg tid	oral	3 days/week one week out of two

the treatment in the form of a bolus with 5-FU being repeated every week after the D_1-D_5 induction phase, for 52 weeks; that with an isoflavonoid being repeated at the same rate, the day of the 5-FU bolus and then the next 2 days.

- **for the treatment of colorectal cancer which is refractory to treatment with 5-fluorouracil (5-FU) and with metastases:**
- according to M.L. Rothenberg et al. (J. Clin. Oncol. 1996; 14: 1128-1135):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3, D_8-D_{10}, D_{15}-D_{17}, D_{22}-D_{24}
• irinotectan	125 mg/m²/day	i.v.	D_1, D_8, D_{15}, D_{22}

the cure comprising two cycles, 42 days apart.

13/ <u>Kaposi's sarcomas</u>

- the isoflavonoids may be combined with the two protocols using antracyclines formulated in the form of liposomes:

i) protocol described by P.S. Gill et al. (J. Clin. Oncol. 1995; 13: 996-1003) and C.A. Presant et al. (Lancet 1993; 341: 1242-1243):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3 and D_{15}-D_{17}
• liposomal daunorubicin	20 mg/m²/day infusion of 1 hour	i.v.	D_1, D_{15}

the cure comprising two cycles repeated at an interval of 28 days before evaluating the effects.

ii) protocol of M. Harrison et al. (J. Clin. Oncol. 1995; 13: 914-920):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3
• liposomal doxorubicin	20 mg/m² infusion of 30 minutes	i.v.	D_1

the cure comprising two cycles repeated at an interval of 28 days before evaluating the effects.

14/ Metastatic melanomas

- The isoflavonoids may also be incorporated into the combined protocols for treating metastatic malignant melanomas:
- DTIC/TAM protocol: according to G. Cocconi et al. (N. Eng. J. Med. 1992; 327: 516), the cure comprising the repetition of 4 cycles, at the rate of 1 cycle every 21 days, according to the following scheme:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• dacarbazine (DTIC)	250 mg/m²/day infusion [15 to 30 min if central catheter] or [30 min if peripheral infusion in 250 ml]	i.v.	D_1-D_5
• tamoxifen (TAM)	20 mg/m²/day	oral	D_1-D_5

the cure comprising 4 cycles at the rate of 1 cycle every 21 days.

15/ Neuroendocrine carcinoma

- the isoflavonoids may be combined with the protocol described by C.G. Moertel et al. (Cancer 1991; 68: 227):
- Pt-E protocol:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3
• etoposide	130 mg/m²/day infusion of 1 hour	i.v.	D_1-D_3
• cisplatin	45 mg/m²/day infusion of 1 hour	i.v.	D_2, D_3

the cure comprising two cycles repeated every 28 days.

16/ Pancreatic cancer

- **advanced-stage pancreatic adenocarcinoma:** the isoflavonoids may be combined with the treatment with gemcitabine according to the protocol of M. Moore et al. (Proc. Am. Soc. Clin. Oncol. 1995; 14: 473):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m^2/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3, D_{8-10}, D_{15}, D_{22}, D_{29}, D_{36}, D_{43}, D_{57}
• gemcitabine	1000 mg/m^2 infusion of 0.5 hour	i.v.	D_1, D_8, D_{15}, D_{22}, D_{29}, D_{36}, D_{43}, then D_{57} then once/week for 3 weeks then 1 week rest and evaluation

B. Oncohaematology

1/Acute adult leukaemias

1.1. Acute lymphoblastic leukaemia:

1.1.1. Linker protocol

The isoflavonoids may be added to the Linker protocols - induction chemotherapy and consolidation chemotherapy (see C.A. Linker et al. Blood 1987; 69: 1242-1248 and C.A. Linker et al. Blood 1991; 78: 2814-2822) according to the following schemes:

i) induction chemotherapy:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m^2/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}, D_{15}-D_{19}

• daunorubicin	50 mg/m² bolus every 24 hours (30 mg/m² in patients of over 50 years)	i.v.	D_1, D_2, D_3
• vincristine	2 mg bolus	i.v.	D_1, D_8, D_{15}, D_{22}
• prednisone	60 mg/m²/day	oral	D_1-D_{28}
• L-asparaginase	6000 U/m²	i.m.	D_{17}-D_{28}

ii) consolidation chemotherapy (regime A):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}
• daunorubicin	50 mg/m² bolus every 24 hours	i.v.	D_1, D_2
• vincristine	2 mg bolus	i.v.	D_1, D_8,
• prednisone	60 mg/m²/day divided into 3 doses	oral	D_1-D_{14}
• L-asparaginase	12,000 U/m²	i.m.	D_2, D_4, D_7, D_9 and D_{14}

the consolidation cure A comprises 4 consecutive cycles as that described above = cycles 1, 3, 5 and 7.

iii) consolidation chemotherapy (regimes B and C):
The regimes described below correspond to the consolidation cycles 2, 4, 6 and 8 (regime B) and 9 (regime C), described by C.A. Linker et al.:

regime B:	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day <u>or</u> 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}
• Ara-C	300 mg/m² infusion of 2 hours	i.v.	D_1, D_4, D_8, D_{11}

• teniposide	165 mg/m² infusion of 2 hours (4 cycles)	i.v.	D_1, D_4, D_8, D_{11}

regime C:	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• methotrexate	690 mg/m² continuous infusion of 42 hours	i.v.	D_1-D_2
• leucovorin	15 mg/m² every 6 hours	oral	D_2-D_5

1.1.2. Hoelzer protocol

The claimed products may be added to the cytotoxic agents of this polychemotherapy protocol (D. Hoelzer et al., Blood 1984; 64: 38-47, D. Hoelzer et al., Blood 1988; 71: 123-131) according to the following scheme:

i) induction chemotherapy/Phase 1:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12} D_{15}-D_{19}
• daunorubicin	25 mg/m²	i.v.	D_1, D_8, D_{15}, D_{22}
• vincristine	1.5 mg/m² (maximum 2 mg)	i.v.	D_1, D_8, D_{15}, D_{22}
• prednisone	60 mg/m²	oral	D_1-D_{28}
• L-asparaginase	5000 U/m² (maximum 2 mg)	i.m.	D_1-D_{14}

ii) induction chemotherapy/phase 2:

The phase 2 of the induction may be carried out as follows:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_{29}-D_{33}, D_{36}-D_{40}, D_{43}-D_{47}
• cyclo-phosphamide	650 mg/m² (maximum 1000 mg)	i.v.	D_{29}, D_{43}, D_{57}
• cytarabine	75 mg/m²/day infusion of 1 h	i.v.	D_{31}-D_{34}, D_{38}-D_{41}, D_{45}-D_{48}, D_{52}-D_{55}
• mercapto-purine	60 mg/m²	oral	D_{29}-D_{57}
• methotrexate	10 mg/m²/day (maximum 15 mg)	i.v.	D_{31}, D_{38}, D_{45}, D_{52}

iii) reinduction chemotherapy/phase 1:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}, D_{15}-D_{19}, D_{22}-D_{26}
• doxorubicin	25 mg/m²/day	i.v.	D_1, D_8, D_{15}, D_{22}
• dexamethasone	10 mg/m²/day	oral	D_1-D_{28}
• vincristine	1.5 mg/m²/day (maximum 2 mg)	oral	D_1, D_8, D_{15} and D_{22}

iv) reinduction chemotherapy/phase 2:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_{31}-D_{35}, D_{38}-D_{42}
• cyclophos-phamide	650 mg/m² (maximum: 1000 mg)	i.v.	D_{29}
• cytarabine	75 mg/m²	i.v.	D_{31}-D_{34}, D_{38}-D_{41}
• thioguanine	60 mg/m²	oral	D_{29}-D_{42}

1.2. Acute myeloid leukaemias:

1.2.1. Treatment of adults of any age

The isoflavonoids may be added, according to the scheme below, to the treatment incorporating the standard dose of cytarabine previously described by R.O. Dilleman et al. (Blood, 1991; 78: 2520-2526), Z.A. Arlin et al. (Leukemia 1990; 4: 177-183) and P.H. Wiernik et al. (Blood 1992; 79: 313-319):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_{12}
• cytarabine	100-200 mg/m²/day as a continuous infusion	i.v.	D_1-D_7
• daunorubicin	45 mg/m²/day as a bolus (30 mg/m²/day if age ≥ 60)	i.v.	D_1-D_3, or D_8-D_{10}
or			
• mitoxantrone	12 mg/m² as a daily bolus	i.v.	D_1-D_3
or			
• idarubicin	13 mg/m² as a daily bolus	i.v.	D_1-D_3

1.2.2. Treatment of adults below 60 years of age

i) induction chemotherapy:

This induction cycle incorporates the administration of cytarabine in a high dose according to the following scheme:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_{10}

• Ara-C (cytarabine)	2000 mg/m²/day as an infusion of 2 hours, every 12 hours	i.v.	D_1-D_6
• daunorubicin or • cytarabine	60 mg/m²/day as a continuous infusion of 24 hours 3000 mg/m²/day as an infusion of 1 hour, every 12 hours	i.v. i.v.	D_4-D_6 D_1-D_6
• daunorubicin	45 mg/m² bolus every 24 hours	i.v.	D_7-D_9

(in order to reduce the risk of S.N.S. toxicity, in the event of renal insufficiency, adjust the cytarabine dosage to the clearance of creatinine)
according to L.E. Damon et al. (Leukemia 1994; 8: 535-541), G.L. Phillips et al. (Blood 1991; 77: 1429-1435) and G. Smith et al. (J. Clin. Oncol. 1997; 15: 833-839).

ii) consolidation chemotherapy:
The cycle, described below, will be repeated 8 times, at the rate of 1 cycle every 4 to 6 weeks (according to R.J. Mayer et al., N. Engl J. Med. 1994; 331: 896-903):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5

• cytarabine	3000 mg/m² as an infusion of 3 hours, every 12 hours (4 cycles)	i.v.	D_1, D_3, D_5
then • cytarabine	100 mg/m²/day every 12 hours	s.c.	D_1-D_5
• daunorubicin	45 mg/m² bolus (4 cycles)	i.v.	D_1

iii) consolidation chemotherapy (with a high dose of cytarabine):
The cycle, described below, will have to be repeated twice and is adapted according to G.L. Phillips et al, (Blood 1991; 77: 1429-1435); S.N. Wolff et al, (J. Clin. Oncol. 1989; 7: 1260-1267); R.J. Mayer et al. (N. Engl J. Med. 1994; 331: 896-903):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_{10}
• cytarabine	3000 mg/m² 1 hour every 12 hours	i.v.	D_1-D_6
• daunorubicin	30-45 mg/m²/day bolus once/day	i.v.	D_7-D_9

1.2.3. Treatment of adults aged 60 or above

The claimed substances may be added to the consolidation chemotherapy protocols below:

i) according to R.O. Dilman et al, (Blood 1991; 78; 2520-2526), Z.A. Arlin et al. (Leukemia 1990; 4: 177-183), P.H. Wiernik et al. (Blood 1992; 79: 313-319):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_6
• cytarabine	100-200 mg/m² continuous infusion of 24 hours	i.v.	D_1-D_5
• daunorubicin	30-45 mg/m²/day bolus	i.v.	D_1, D_2,
or • mitoxantrone	12 mg/m²/day bolus	i.v.	D_1, D_2
or • idarubicin	13 mg/m²/day bolus	i.v.	D_1, D_2

ii) according to R.J. Mayer et al. (N. Engl. J. Med, 194; 331: 896-903):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_6
• cytarabine	100 mg/m² continuous infusion of 24 hours (4 cycles)	i.v.	D_1-D_5
then • cytarabine	100 mg/m² every 12 hours	s.c.	D_1, D_5
• daunorubicin	45 mg/m²/day bolus (4 cycles)	i.v.	D_1

iii) according to C.A. Linker et al. (Blood 1993; 81: 311-318), N. Chao et al. (Blood 1993; 81: 319-323) and A.M. Yeager et al. (N. Eng. J. Med. 1986; 315: 145-147):

This protocol comprises an autologous bone marrow transplant (performed on day D_0):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_{-7}-D_{-2}
• busulfan	1 mg/kg qid (in total 16 doses)	oral	D_{-7} to D_{-4}
• etoposide	60 mg/kg/day infusion of 10 hours	i.v.	D_{-3}

or

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_{-9}-D_{-1}
• busulfan	1 mg/kg qid	oral	D_{-9} to D_{-6}
• cyclo-phosphamide	50 mg/kg/day infusion of 1 hour	i.v.	D_{-5} to D_{-2}

iv) in the case of HLA-compatible allogeneic bone marrow transplant according to:

P.J. Tutscha et al. Blood 1987; 70: 1382-1388, F.R. Applebaum et al., Ann. Int. Med. 1984; 101: 581-588:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_{-7}-D_{-1}

• busulfan	1 mg/kg qid (in total 16 doses)	oral	D_{-7} to D_{-4}
• cyclo-phosphamide	60 mg/kg/day infusion of 1 hour	i.v.	D_{-3} to D_{-2}

2/ Chronic adult leukaemias

2.1 Chronic myeloid leukaemia

In the myeloblastic phase, the isoflavonoids may be added to the HU-Mith treatment, described by C.A. Koller et al. (N. Engl. J. med. 1986; 315: 1433-1438):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 D_8-D_{12} D_{15}-D_{19} D_{22}-D_{26}
• hydroxyurea	500 mg/day	oral	every day
• mithramycin	25 µg/kg/day infusion of 2-4 hours	i.v.	daily for 3 weeks then 3 times/week

2.2 Chronic lymphocytic leukaemia

2.2.1 FCG-CLL protocol

The isoflavonoids may be added to the "pulsed chlorambucil" combinations as described by E. Kimby et al. (Leuk. Lymphoma 1991; 5 (Suppl.) 93-96) and by FCGCLL (Blood 1990; 75: 1422-1425):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_{-8}-D_{-12}, D_{15}-D_{22}

• chlorambucil	0.1 mg/kg/day	oral	once/day
or			
• chlorambucil	0.4 mg/kg/day every 14 days	oral	D_1
and			
• prednisone	75 mg/day	oral	D_1-D_3

2.2.2 Fludarabine-CdA protocol

according to H.G. Chun et al. (J. Clin. Oncol. 1991; 9: 175-188), M.J. Keating et al. (Blood 1989; 74: 19-25 / J. Clin. Oncol. 1991; 9: 44-49) and A. Saven et al. (J. Clin. Oncol. 1995; 13: 570-574):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m^2/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_8 (once/month for 6 to 12 cycles)
• fludarabine	25-30 mg/m^2/day infusion of 30 minutes [every 4 weeks for 6 to 12 cycles]	i.v.	D_1-D_5
or			
• cladibrine	0.09 mg/kg/day as a continuous infusion [1 cycle every 28 to 35 days for 1 to 9 cycles (median: 4 cycles)]	i.v.	D_1-D_7

3/ Lymphoproliferative diseases

3.1 Hodgkin's disease

The isoflavonoids may be incorporated into the polychemotherapy protocols conventionally used for the treatment of Hodgkin's lymphoma:

3.1.1 AVDB protocol

according to G. Bonnadonna et al. (Cancer Clin. Trials 1979; 2: 217-226) and G.P. Canellos et al. (N. Engl. J. Med. 1993; 327: 1478-1484):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3, D_{15}-D_{18}
• doxorubicin (A)	25 mg/m² bolus	i.v.	D_1, D_{15}
• bleomycin (B)	10 U/m² bolus	i.v.	D_1, D_{15}
• vinblastine (V)	6 mg/m² bolus	i.v.	D_1, D_{15}
• dacarbazine (D)	375 mg/m² bolus	i.v.	D_1, D_{15}

the cure comprising 6 to 8 cycles, at the rate of 1 cycle every 28 days.

3.1.2 MOPP/ABVD protocol

according to G. Bonnadonna et al. (Ann. Intern. Med. 1986; 104: 739-746) and G.P. Canellos et al. (N. Engl. J. Med. 1993; 327: 1478-1484):

The MOPP protocol should be alternated with the ABVD protocol (cf. § 3.1.1) every 28 days and the cure comprises 6 cycles:

MOPP protocol:	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3, D_8-D_{11} and D_{14}-D_{17}

• mechlorethamine (M)	6 mg/m^2 bolus	i.v.	D_1, D_8
• vincristine (O)	1.4 mg/m^2 bolus (no maximum)	i.v.	D_1, D_8
• procarbazine (P)	100 mg/m^2/day	oral	D_1-D_{14}
• prednisone (P)	40 mg/m^2/day	oral	D_1-D_{14}

3.1.3 Stanford V protocol

according to N.L. Bartlett et al. (J. Clin. Oncol. 1995; 13: 1080-1088):

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	Dose	Route	Days
• isoflavonoid	200-2000 mg/m^2/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 D_8-D_{12} D_{15}-D_{19} D_{22}-D_{26}
• doxorubicin	25 mg/m^2	i.v.	D_1, D_{15}
• vinblastine	6 mg/m^2 bolus (4 mg/m^2 during cycle 3 if age ≥ 50)	i.v.	D_1, D_{15}
• mechlorethamine (M)	6 mg/m^2 bolus	i.v.	D_1
• vincristine	1.4 mg/m^2 bolus (max. dose: 2 mg) [1 mg/m^2 during cycle 3 if age ≥ 50)	i.v.	D_1, D_{22}
• bleomycin	5 U/m^2	i.v.	D_8, D_{22}
• etoposide	60 mg/m^2	oral	D_{15}, D_{16}
• prednisone	40 mg/m^2/day	oral	once/week (weeks 1-9)

the cure comprising 3 cycles, at the rate of 1 cycle every 28 days.

3.1.4 EVA protocol

according to G.P. Canellos et al. (Proc. Am. Soc. Clin. Oncol. 1991; 10: 273):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• etoposide (E)	100 mg/m² infusion of 2 hours	oral	D_1, D_2, D_3
• vinblastine (V)	6 mg/m² bolus	i.v.	D_1
• doxorubicin (A)	50 mg/m² bolus	i.v.	D_1

the cure comprising 6 cycles, at the rate of 1 cycle every 28 days.

3.1.5 B-CAVe protocol

according to W.G. Harker et al. (Ann. Intern. Med. 1984; 101: 440-446):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_3
• bleomycin (B)	5 U/m² bolus	i.v.	D_1
• lomustine (CCNU)	100 mg/m²	oral	D_1
• doxorubicin (A)	60 mg/m² bolus	i.v.	D_1
• vinblastine (Ve)	5 mg/m² bolus	i.v.	D_1

the cure comprising 8 cycles, at the rate of 1 cycle every 28 days.

3.2. Non-Hodgkin's lymphomas

3.2.1. of low grade of malignancy

i)-CVP protocol

- according to C.M. Bagley et al. (Ann. Intern. Med. 1972; 76: 227-234) and C.S. Portlock et al. (Blood 1976; 47: 747-756)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• cyclophosphamide (c)	300-400 mg/m²/day	oral	D_1, D_5
• vincristine (V)	1.4 mg/m² bolus (max: 2 mg)	i.v.	D_1
• prednisone (P)	100 mg/m² day	oral	D_1-D_5

This cycle is repeated every 21 days up to the maximum response

ii)- I-COPA protocol

- according to RV Smalley et al. (N. Eng. J. Med. 1992; 327: 1336-1341)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• cyclophosphamide (C)	600 mg/m²/day	i.v.	D_1
• vincristine (O)	1.2 mg/m² bolus (max: 2 mg)	i.v.	D_1
• prednisone (P)	100 mg/m²/day	i.v.	D_1-D_5
• doxorubicin (A)	50 mg/m² bolus	i.v.	D_1
• interferon-alpha (I)	6 MU/m²	i.m.	D_{22}-D_{26}

The cure comprises 8 to 10 cycles, at the rate of one cycle every 28 days.

iii)- Fludarabine-CdA protocol

- according to P. Solol-Celigny et al. (Blood 1994; 84 (Supp. 1): 383a), H. Hoeschster et al.; (Blood 1994; 84 (Suppl. 1): 564a and A.C. Kay (J. Clin. Oncol. 1992; 10: 371-377)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_7
• fludarabine	25 mg/m²/day infusion of 0.5 hour	i.v.	D_1-D_5
or • fludarabine	20 mg/m²/day	i.v.	D_1-D_5
and cyclophos- phamide	600 - 1000 mg/m²/day	i.v.	D_1
or cladribine	0.1 mg/m²/day infusion of 24 hours	i.v.	D_1-D_7

For fludaribine, each cycle is repeated every 28 days; for cladribine, each cycle is repeated every 35 days.

3.2.2. of intermediate malignancy grade

i)-CHOP or CNOP protocol

- according to EM McKelvey et al. (Cancer 1976; 38: 1484 - 1493), J.O. Armitage et al. (J. Clin. Oncol. 1984; 2: 898-902, S. Paulovsky et al. (Ann. Oncol. 1992; 3: 205-209)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• cyclophosphamide (c)	750 mg/m²/day	i.v.	D_1
• doxorubicin (H)	50 mg/m² bolus	i.v.	D_1
• vincristine (O)	1.4 mg/m² bolus (max. 2 mg)	i.v.	D_1

• prednisone (P)	100 mg/m²/day (as 1 dose/day)	oral	D_1-D_5

for the CHOP protocol

The mitoxantrone (N) may be used to replace (CNOP protocol) the doxorubicin in patients over 60 (dose: 12 mg/m² as an i.v. bolus on day D1 of each cycle).

The cure by the CHOP or CNOP protocol comprises 6 to 8 cycles at the rate of 1 cycle every 21 days.

ii) - MACOP-B protocol

- according to P. Klimo et al. (Ann. Intern. Med. 1985; 102: 596-602) and I.A. Cooper et al. (J. Clin. Oncol. 1994; 12: 769-778)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}, D_{15}-D_{22}, D_{29}-D_{33} D_{43}-D_{47}, D_{57}-D_{61} D_{71}-D_{75}
• methotrexate (M)	100 mg/m²/bolus then 300 mg/m² infusion of 4 hours	i.v.	D_8, D_{36}, D_{64}
• leucovorin	15 mg qid	oral	D_9, D_{37}, D_{65}
• doxorubicin (A)	50 mg/m² bolus	i.v	D_1, D_{15}, D_{29}, D_{43}, D_{57}, D_{71}
• cyclo-phospshamide (c)	350 mg/m² bolus	i.v.	D_1, D_5, D_{29}, D_{43}, D_{57}, D_{71}
• vincristine (D)	1.4 mg/m² bolus (max: 2 mg)	i.v.	D_8, D_{22}, D_{36}, D_{50}, D_{64}, D_{78}

• prednisone (P)	75 mg/day	oral	Every day for 12 weeks
• bleomycin	10 U/m^2 bolus	i.v.	D^{22}, D^{50}, D^{78}

This treatment protocol extends over 12 weeks and corresponds to 1 cycle.

iii) - VACOP-B protocol

- according to J.M. Connors et al. (Proc. Am. Soc. Clin. Oncol. 1990; 9:254):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m^2/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}, D_{15}-D_{22}, D_{29}-D_{34}, D_{43}-D_{47}, D_{57}-D_{61}, D_{71}-D_{75}
• etoposide (V)	50 mg/m^2	i.v.	D_{15}, D_{43}, D_{71}
• etoposide	100 mg/m^2	oral	D_{16}, D_{17}, D_{44}, D_{45}, D_{72}, D_{73}
• doxorubicin (A)	50 mg/m^2 bolus	i.v.	D_1, D_{15}, D_{29}, D_{43}, D_{57}, D_{71}
• cyclophosphamide (c)	30 mg/m^2 day bolus	i.v.	D_8, D_{22}, D_{36} D_{50}, D_{64}, D_{78}
• vincristine (O)	1.2 mg/m^2 bolus	i.v.	D_8, D_{22}, D_{36}, D_{50}, D_{64}, D_{78}

• prednisone (P)	45 mg/m²/day	oral	1/day for 1 week, then 4/day the next 11 weeks

Each cycle lasting for 12 weeks.

iv)- m-BACOD/M-BACOD protocol

- according to M.A. Shipp et al. (Ann. Int. Med. 1986; 140: 757-765) and A.T. Skarin et al. (J. Clin. Oncol. 1983; 1:91-98)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}, D_{15}-D_{19}
• methotrexate (m)	200 mg/m² infusion of 4 hours	i.v	D_8, D_{15}
or (M)	3000 mg/m² infusion of 4 hours	i.v.	or D_{15}
• leucovorin	10 mg/m² qid (6 doses in total)	oral	D_9, D_{16} or D_{16}
• bleomycin (B)	4 U/m² bolus	i.v.	D_1
• doxorubicin (A)	45 mg/m² day bolus	i.v.	D_1
• cyclophosphamide (C)	600 mg/m² bolus	i.v.	D_1
• vincristine (O)	1 mg/m² bolus	i.v.	D_1
• dexamethasone (D)	6 mg/m²/day	oral	D_3-D_5

The cure comprising 10 cycles, at the rate of 1 cycle every 21 days.

v)- ProMACE/CytaBOM protocol

- according to D.L. Longo et al. (J. Clin. Oncol. 1991; 9: 25-38):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}
• cyclophosphamide (C)	650 mg/m² infusion of 0.5 hour	i.v.	D_1
• doxorubicin (A)	25 mg/m² bolus	i.v.	D_1
• etoposide	120 mg/m² infusion of 1 hour	i.v.	D_1
• prednisone (P)	60 mg/day	oral	D_1-D_{14}
• cytarabine	300 mg/m² bolus	i.v	D_8
• bleomycin (B)	5 U/m² bolus	i.v	D_8
• vincristine (O)	1.4 mg/m² bolus	i.v	D_8
• methotrexate	120 mg/m² bolus	i.v	D_8
• leucovorin	25 mg/m² qid (4 doses in total)	oral	D_9

The cure comprising 6 to 8 cycles, at the rate of one cycle every 14 days.

3.2.3. of low or intermediate malignancy grade

i)- ESHAP rescue protocol

- in case of recidivation or in case of failure of the first line treatment, according to W.S. Velasquez et al. (J. Clin. Oncol. 1994; 12: 1169-1176)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• etoposide (E)	40 mg/m² infusion of 2 hours	i.v.	D_1-D_4
• methyl-prednisolone (S)	500 mg/day infusion of 15 minutes	i.v.	D_1, D_4

• cytarabine (HA)	2000 mg/m² infusion of 3 hours	i.v.	D_5
• cisplatin (P)	25 mg/m²/day bolus continuous infusion of 24 hours	i.v.	D_1-D_4

The cure comprising 6 cycles, at the rate of 1 cycle every 28 days.

ii)- MINE rescue protocol

- in case of recidivation or in the case of failure of the first line treatment, according to F. Cabanillas et al. (Semin. Oncol. 1990; 17 (Suppl. 10): 28-33)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• ifosfamide (I)	1330 mg/m² infusion of 1 hour	i.v.	D_1-D_3
• mesna (M)	1330 mg/m² in the ifosfamide infusion then 266 mg/m² bolus 4 and 8 hours after each dose of ifosfamide	i.v.	D_1-D_3
• mitoxantrone (M)	8 mg/m² infusion of 15 minutes	i.v.	D_1
• etoposide (E)	65 mg/m²/day infusion of 1 hour	i.v.	D_1-D_3

This cycle to be repeated every 21 days.

3.3. Non-Hodgkin's lymphomas: Burkitt's lymphoma, small cell lymphoma, lymphoblastic lymphoma

3.3.1 Magrath protocol

- The claimed products may be combined with the Magrath protocols according to the following schemes:

i) - cycle 1

- according to I.T. Magrath et al. (Blood 1984; 63: 1102-1111)

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}
• cytarabine	30 mg/m²	intra-thecal	D_1, D_2, D_3, D_7
• cyclophosphamide	1200 mg/m² bolus	i.v.	D_1
• methotrexate	12.5 mg/m² (max: 12.5 mg)	intra-thecal	D_{10}
• methotrexate	300 mg/m²/day infusion of 1 hour then 60 mg/m²/h infusion of 41 hours	i.v.	D_{10}-D_{11}
• leucovorin	15 mg/m² bolus qid (8 successive doses)	i.v.	to be started 42 hours after the start of the administration of methotrexate

ii) - cycles 2 to 15

- according to I.T. Magrath et al. (1984) also

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 D_{10}, D_{11}
• cytarabine	45 mg/m²	intra-thecal	D_1-D_2 (cycles 2 and 3) D_1 (cycles 4 and 6)
• cyclophosphamide (C)	1200 mg/m² bolus	i.v.	D_1
• doxorubicin	40 mg/m² bolus	i.v.	D_1
• vincristine	1.4 mg/m² bolus (max: 2 mg)	i.v.	D_1
• methotrexate	12.5 mg/m² (max: 12.5 mg)	intra-thecal	D_3, D_{10} (cycles 2 and 3) D_{10} (cycles 4, 5, 6)
• methotrexate	300 mg/m² infusion of 1 hour then 60 mg/m² continuous infusion of 41 hours	i.v.	D_{10}, D_{11} (cycles 2 and 6) D_{14}, D_{15} (cycles 7-15)
• leucovorin	15 mg/m² bolus qid (8 consecutive doses)	i.v.	start at the 42nd hour of the treatment with metho-trexate

the cure comprising 14 cycles, at the rate of one cycle every 28 days.

3.4 Waldenström macroglobulinaemia

3.4.1 CVP protocol

according to the CVP protocol described by M.A. Dimopoulous et al, (Blood 1994; 83: 1452-1459) and C.S. Portlock et al. (Blood 1976; 47: 747-756):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• cyclo-phosphamide (C)	300-400 mg/m²/day	oral	D_1-D_5
• vincristine (V)	1.4 mg/m²/day bolus (max: 2 mg)	i.v.	D_1
• prednisone (P)	100 mg/m²/day	oral	D_1-D_5

the cure to be continued indefinitely (1 cycle every 21 days).

3.4.2 Fludarabine-CdA protocol

according to H.M. Kantarjian et al. (Blood 1990; 75: 1928-1931) and M.A. Dinopoulous et al. (Ann. Intern. Med. 1993; 118: 195-198):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• fludarabine	25-30 mg/m² infusion of 0.5 hour	i.v.	D_1-D_5

or

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_7
• cladribine (CdA)	0.09 mg/m²/day continuous infusion	i.v.	D_1-D_7

the cure comprising 6 to 12 cycles 28 days apart in the case of fludarabine and 2 cycles 28 days apart also in the case of cladribine.

3.5 Multiple myeloma

3.5.1 MP protocol

according to R. Alexanian et al. (JAMA 1969: 208: 1680-1685), A. Belch et al. (Br. J. Cancer 1988; 57: 94-99) and F. Mandelli et al. (N. Engl. J. med. 1990; 322: 1430-1434):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• melphalan (M)	0.25 mg/kg/day	oral	D_1-D_4
• prednisone (P)	100 mg/day	oral	D_1-D_4

or

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• melphalan (M)	9 mg/m²/day	oral	D_1-D_4
• prednisone (P)	100 mg/day	oral	D_1-D_4

the cure comprising at least 12 cycles, at the rate of 1 cycle every 4 to 6 weeks.

3.5.2 VAD protocol

according to B. Barlogie et al, (N. Engl. J. Med. 1984; 310: 1353-1356):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• vincristine (V)	0.4 mg/day continuous infusion of 24 hours	i.v.	D_1-D_4

• doxorubicin (A)	9 mg/m²/day continuous infusion of 24 hours	i.v.	D_1-D_4
• dexamethasone (D)	40 mg/day	i.v.	D_1-D_4, D_9-D_{12}, D_{17}-D_{20}

3.5.3 MP-interferon α protocol according to O. Osterborg et al. (Blood 1993; 81: 1428-1434):

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• melphalan (M)	0.25 mg/kg/day	oral	D_1-D_4
• prednisone (P)	2 mg/kg/day	oral	D_1-D_4
• interferon-alpha	7 MU/m²/day	s.c.	D_1-D_5 and D_{22}-D_{26}

the cure comprising the indefinite repetition of this cycle, at the rate of 1 cycle every 42 days.

3.5.4 VCAP or VBAP protocol according to S.E. Salmon et al. (J. Clin. Oncol. 1983; 1: 453-461):

VCAP protocol:

	Dose	Route	Days
• isoflavonoid	200-2000 mg/m²/day or 5 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5
• vincristine (V)	1 mg/m² bolus (max: 1.5 mg)	i.v.	D_1
• doxorubicin	30 mg/m² bolus	i.v.	D_1
• prednisone (P)	60 mg/m²/day	oral	D_1-D_4
• cyclophosphamide (C)	125 mg/m²	oral	D_1-D_4

VBAP protocol: the cyclophosphamide is replaced with carmustine (BCNU), the remainder being identical:

	Dose	Route	Days
• carmustine	30 mg/m² infusion of 1 hour	i.v.	D_1

C. CHILDHOOD TUMOURS - Paediatric oncology

The isoflavonoids may also be incorporated into the polychemotherapy protocols for treating paediatric tumours in order to enhance the antitumour efficacy while reducing the severity of the side effects by means of the action on the recruitment and mobilization of clonogenic cells and the possibility of reducing the active doses.

1/ Ewing sarcoma/primitive neuroectodermal tumour

The isoflavonoids may be introduced in the VCR-Doxo-CY-Ifos-Mesna-E (E.D. Bergert et al., J. Clin. Oncol. 1990; 8: 1514-1524; W.H. Meyer et al., J. Clin. Oncol. 1992; 10: 1737-1742):

	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{11}, D_{15}-D_{18}, D_{22}-D_{27},
• vincristine	2 mg/m² bolus (maximum dose = 2 mg)	i.v.	D_1, D_8, D_{15}, D_{43}
• doxorubicin	30 mg/m²/day as an infusion of 24 hours	i.v.	D_1-D_3, D_{43}-D_{45}
• cyclo-phosphamide	2.2 g/m² as an infusion of 0.5 hour	i.v.	D_1, D_{43}
• ifosfamide	1800 mg/m²/day as an infusion of 1 hour	i.v.	D_{22}-D_{26} D_{63}-D_{67}

• mesna	360 mg/m² as an infusion of 15 minutes at the rate of 5 doses every 3 hours	i.v.	administered with cyclophosphamide and ifosfamide
• etoposide	100 mg/m² as an infusion of 1 hour	i.v.	D_{22}-D_{26} D_{63}-D_{67}

the cure comprises 6 to 10 of these cycles depending on the initial severity of the sarcoma and the extent of the response.

2/ Childhood acute lymphoblastic leukaemia

2.1. Induction chemotherapy (days D_1-D_{-30})

The isoflavonoids may be added to the recommended protocols (P.S. Gaynon et al., J. Clin. Oncol., 1993, 11, 2234-2242; J. Pullen et al., J. Clin. Oncol. 1993; 11: 2234-2242; J. Pullen et al., J. Clin. Oncol. 1993; 11: 839-849; VJ Land et al., J. Clin. Oncol. 1994; 12: 1939-1945):

	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 and D_{22}-D_{27} and D_1, D_8, D_{15} and D_{22}
• vincristine	1.5 mg/m² bolus (maximum dose = 2 mg)	i.v.	D_1, D_8, D_{15}, D_{22}
• L-asparaginase	6000 IU/m²	i.m.	3 times/week for 3 weeks
• prednisone	60 mg/m² in 3 doses/day	oral	D_1 to D_{28}
• daunorubicin	25 mg/m²day as an infusion of 15 minutes	i.v.	D_1, D_8, D_{15} and D_{22}
• methotrexate	depending on age the	intra-thecal	D_{15}, D_{28}

• cytarabine	depending on age	intra-thecal	D_1

depending on the result of the examination of the bone marrow, the passage to the consolidation phase is made on day D_{28} of the treatment protocol.

2.2. Consolidation/maintenance chemotherapy

The isoflavonoids may be introduced in the maintenance protocol (P.S. Gaynon et al., J. Clin. Oncol. 1993; 11: 2234-2242; J. Pullen et al., J. Clin. Oncol. 1993; 11: 839-849; V.J. Land et al., J. Clin. Oncol. 1994; 12: 1939-1945) according to the following scheme:

	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 hour	i.v.	D_1-D_5, D_{15}-D_{20} and D_{94}-D_{99}, D_{101}-D_{106}, D_{108}-D_{113}, D_{122}-D_{127}
• cyclophos-phamide	1000 mg/m² as an infusion of 0.5 hour	i.v.	D_1, D_{15}, D_{122}
• L-asparaginase	6000 U/m²	i.m.	3 times/week between D_{97} and D_{122}
• cytarabine	75 mg/m²/day as an infusion of 15 minutes	i.v./s.c.	a sequence of 4 days starting D_2, D_9, D_{16}, D_{23}, D_{123}, D_1
• doxorubicin	25 mg/m²/day as an infusion of 15 minutes	i.v.	D_{94}, D_{101}, D_{108}
• mercaptopurine	60 mg/m²/day	oral	D_1-D_{93}, D_{143} at the end of the treatment

• methotrexate	20 mg/m²/day	oral	once/week between D_{36} and D_{72} and between D_{143} and the end of the treatment
• prednisone	40 mg/m²/day (divided into 3 doses/day)	oral	5 consecutive days per month between D_{143} and the end of the treatment
• thioguanine	60 mg/m²/day	oral	D_{122}-D_{135}
• vincristine	1.5 mg/m² bolus (maximum dose = 2 mg)	i.v.	D_{94}, D_{101}, D_{108}, then once/month between D_{143} and the end of the treatment
• methotrexate	depending on age	intra-thecal	D_1, D_8, D_{15}, D_{22}, D_{123}, D_{130} then once/3 months between D_{143} and the end of the treatment

3/ Childhood acute myeloid leukaemia

The isoflavonoids are added to the induction and consolidation/maintenance protocols according to the following schemes:

3.1. Induction chemotherapy

According to Y. Ravindranath et al., J. Clin. Oncol. 1991; 9: 572-580; M.E. Nesbit et al., J. Clin.

Oncol. 1994; 12: 127-135; RJ Wells et al., J. Clin. Oncol. 1994; 12: 2367-2377):

	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_{10}-D_{13}
• cytarabine	according to age	intra-thecal	D_1
• daunorubicin	20 mg/m²/day as an infusion of 24 hours	i.v.	D_1-D_4, D_{10}-D_{13}
• cytarabine	200 mg/m²/day as an infusion of 24 hours	i.v.	D_1-D_4, D_{10}-D_{13}
• thioguanine	100 mg/m²/day divided into 2 doses/day	oral	D_1-D_4, D_{10}-D_{13}
• etoposide	100 mg/m²/day as an infusion of 24 hours	i.v.	D_1-D_4, D_{10}-D_{13}
• dexamethasone	6 mg/m² divided into 3 doses/day	i.v./ oral	D_1-D_4, D_{10}-D_{13}

this cycle being repeated from D_{28}.

3.2. Consolidation/maintenance chemotherapy

According to Y. Ravidranath et al., J. Clin. Oncol. 1991; 9: 572-580; M.E. Nesbit et al., J. Clin. Oncol. 1994; 12: 127-135; R. J. Wells et al, J. Clin. Oncol. 1994; 12: 2367-2377):

	Dose	Route	Days
• cytarabine	according to age	intra-thecal	D_1, D_{28}, D_{56}
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{13} and D_{28}-D_{33}, D_{56}-D_{61}, D_{89}-D_{94}

• cytarabine	3000 mg/m² as an infusion of 3 hours every 12 hours	i.v.	D_1-D_2, and D_8-D_9
• L-asparaginase	6000 IU/m² 3 hours after cytarabine	i.m.	D_2, D_9
• vincristine	1.5 mg/m² bolus (maximum dose = 2 mg)	i.v.	D_{28}, D_{56}
• thioguanine	75 mg/m²/day	oral	D_{28}-D_{84}
• cytarabine	25 mg/m²/day bolus	i.v.	D_{28}-D_{31}, D_{56}-D_{59}
• cyclophos-phamide	75 mg/m²/day as an infusion of 0.5 hour	i.v.	D_{28}-D_{31}, D_{56}-D_{59}
• cytarabine	25 mg/m²/day bolus	sc/i.v.	D_{89}-D_{93}
• thioguanine	50 mg/m²/day	oral	D_{89}-D_{93}
• etoposide	100 mg/m²/day as an infusion of 1 hour	i.v.	D_{89}, D_{92}
• dexamethasone	2 mg/m²/day	oral	D_{89}-D_{92}
• daunorubicin	30 mg/m² as an infusion of 15 minutes	i.v.	D_{89}

4/ Childhood Hodgkin's disease

The isoflavonoids may be addeed to the MOPP-ABVD protocol according to EA Gehan et al. (Cancer 1990; 65: 1429-1437), SP Hunger et al. (J. Clin. Oncol. 1994; 12: 2160-2166) and MM Hudson et al. (J. Clin. Oncol. 1993; 11: 100-108):

	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5 and D_8-D_{12}

• mechlorethamine (M)	6 mg/m^2 bolus	i.v.	D_1, D_8
• vincristine (O)	1.5 mg/m^2 bolus (maximum 2 mg)	i.v.	D_1, D_8
• procarbazine (P)	100 mg/m^2/day	oral	D_1-D_{14}
• prednisone (P)	40 mg/m^2/day (divided into 3 doses/d)	oral	D_1-D_{14}
• doxorubicin (A)	25 mg/m^2/day as an infusion of 15 minutes	i.v.	D_{29}, D_{-43}
• bleomycin (B)	10 U/m^2 as an infusion of 15 minutes	i.v.	D_{29}, D_{43}
• vinblastine	6 mg/m^2 bolus (maximum 2 mg)	i.v.	D_{29}, D_{43}
• dacarbazine (D)	375 mg/m^2 as an infusion of 15 minutes	i.v.	D_{29}, D_{43}

This cycle should be repeated 6 times at the rate of 1 cycle every 8 weeks, the cure comprising 6 cycles.

If an autologous bone marrow transplant (autograft) is prescribed, the CVB protocol described by R. Chopra et al. (Blood 1993; 81: 1137-145), C. Wheeler et al. (J. Clin. Oncol. 1990; 8: 648-656) and RJ Jones et al (J. Clin Oncol 1990, 8, 527-537) may be used according to the following scheme (the allograft taking place on day D_0):

	Dose	Route	Days
• isoflavonoid	100-200 mg/m^2/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_{-7}, D_{-1}
• cyclo-phosphamide	1800 mg/m^2/day as 2 infusions of 1 hour	i.v.	D_{-7}, D_{-6} D_{-5}, D_{-4}

• carmustine (BCNU)	112 mg/m²/day as an infusion of 0.5 hour	i.v.	D_{-7}, D_{-6} D_{-5}, D_{-4}
• etoposide	500 mg/m²/day as 2 infusions of 1 hour	i.v.	D_{-7}, D_{-6} D_{-5}, D_{-4}

5/ Childhood lymphoblastic lymphoma

The isoflavonoids may also be combined with the induction chemotherapy protocols (A.T. Meadows et al., J. Clin. Oncol. 1989; 7: 92-99 - C. Patte et al., Med. Ped. Oncol. 1992; 20: 105-113 and A. Reiter et al., J. Clin. Oncol. 1995; 13: 359-372) and the maintenance chemotherapy protocols:

5.1 Induction chemotherapy

	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_{17}-D_{22}, D_{24}-D_{29}
• cyclo-phosphamide	1200 mg/m² as an infusion of 0.5 hour	i.v.	D_1
• cytarabine	according to age	intra-thecal	D_1
• vincristine	1.5 mg/m² bolus (maximum 2 mg)	i.v.	D_3, D_{10}, D_{17}, D_{24}
• prednisone	60 mg/m²/day divided into 3 doses/day	oral	D_3-D_{28}
• daunorubicin	60 mg/m² as an infusion of 15 minutes	i.v.	D_{17}
• L-asparaginase	6000 U/m²/day as an infusion of 15 minutes	im	D_{17}-D_{35} 3 times/week

• methotrexate	according to age	intra-thecal	D_{17}, D_{-31}

5.2 Maintenance chemotherapy

according to the following scheme:

	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_{15}-D_{20}, D_{29}-D_{34}
• cyclo-phosphamide	1000 mg/m² as an infusion of 0.5 hour	i.v.	D_1
• vincristine	1.5 mg/m² bolus (maximum 2 mg)	oral	D_1, D_5, (cycles 2 to 10)
• methotrexate	300 mg/m²/day (60% as an infusion of 15 minutes and 40% as an infusion of 4 hours)	i.v.	D_{15}
• leucovorin	10 mg/m²/every 4 h	oral	D_{16}
• daunorubicin	30 mg/m² as an infusion of 0.5 hour	i.v.	D_{29}
• methotrexate	according to the age	intra-thecal	D_1, D_8, D_{15} (cycle 1), then once/month (cycles 2 to 10)

the cure comprising 10 cycles

6/ Paediatric neuroblastoma

The recommended polychemotherapy Doxo-E-Cy-Pt protocol is adapted from R.P. Castleberry et al. (J. Clin. Oncol. 1992; 10: 1299-1304), A. Garaventa et

al. (J. Clin. Oncol. 1993; 11: 1770-1779) and D.C. West et al. (J. Clin. Oncol. 1992; 11: 84-90):

	Dose	Route	Days
• isoflavonoid	100-200 mg/m^2/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_{28}-D_{35}, D_{58}-D_{65}
• doxorubicin	25 mg/m^2/day as an infusion of 15 minutes	i.v.	D_2, D_{30}, D_{58}
• etoposide	100 mg/m^2 as an infusion of 1 hour	oral/ naso-gastric	D_2, D_5, D_{30}, D_{33}, D_{58}, D_{61}
• cyclo-phosphamide	1000 mg/m^2 as a infusion of 0.5 hour	i.v.	D_3, D_4, D_{31}, D_{32}, D_{59}, D_{60}
• cisplatin	60 mg/m^2 as an infusion of 6 hours	i.v.	D_1, D_{28}, D_{56}

The evaluation of the therapeutic response is made after 9 weeks in order to decide on the attitude: surgical resection, radiotherapy or new chemotherapy.

7/ Paediatric osteosarcoma

The isoflavonoids may be added to the Doxo-Pt-Mtx-Lcv protocol as described by M. Hudson et al. (J. Clin. Oncol. 1990; 8: 1988-1997), PA Meyers (J. Clin. Oncol. 1992; 10: 5-15), and V.H.C. Bramwell et al. (J. Clin. Oncol. 1992; 10: 1579-1591):

	Dose	Route	Days
• isoflavonoid	100-200 mg/m^2/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_{21}-D_{26}, D_{28}-D_{33}
• doxorubicin	25 mg/m^2/day as an infusion of 24 hours	i.v.	D_1-D_3

• cisplatin	120 mg/m² as an infusion of 6 hours	i.v.	D_1
• methotrexate	12 mg/m²/day as an infusion of 1 hour	i.v.	D_{21}, D_{28}
• leucovorin	100 mg/m² every 6 hours	oral	D_{22}, D_{29}

8/ Childhood rhabdomyosarcoma

The Vcr-Dact-CY-Mesna protocol (H. Maurer et al., Cancer 1993; 71: 1904-1922 and LR Mandell et al., Oncology 1993; 7: 71-83) may include i.v. infusion of the isoflavonoids according to the following scheme:

	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}, D_{22}-D_{27}, D_{43}-D_{47}
• vincristine	1.5 mg/m²/day (bolus maximum 2 mg)	i.v.	D_1, D_8, D_{15}, D_{22}, D_{29}, D_{36}, D_{43}, D_{50}, D_{57}
• dactinomycin	0.015 mg/kg bolus (max daily dose: 0.5 mg	i.v.	D_1-D_5, D_{22}-D_{27}, D_{43}-D_{47}
• cyclo-phosphamide	2.2 g/m² as an infusion of 1 hour	i.v.	D_1, D_{22}, D_{43}
• mesna	360 mg/m² as an infusion of 1 hour every 3 hours for 5 doses	i.v.	D_1, D_{22}, D_{43}

At the end of the 9th week of treatment, the efficacy should be evaluated in order to decide on the future course of action (surgery, radiotherapy, continuation of the chemotherapy).

9/ Childhood Wilms tumour

In the Vcr-Dact protocol as described by GJ D'Angio et al. (Cancer, 1989; 64: 349-360) and DM Green et al. (J. Clin. Oncol. 1993; 11: 91-95):

	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_1-D_5, D_8-D_{12}, then every week
• vincristine	2 mg/m² bolus (max dose: 2 mg)	i.v.	D_7 then every week
• dactinomycin	0.045 mg/kg bolus (P≤30 kg) 1.35 mg/m² (P>30 kg) (max dose: 3 mg	i.v.	D_1, then every 3 weeks

This protocol being started after the surgical resection.

In case of autologous bone marrow transplant (autograft) according to A. Garaventar et al. (Med. Pediatr. Oncol. 1994; 22: 11-14), the E-Thio-Cy protocol may be modified as follows

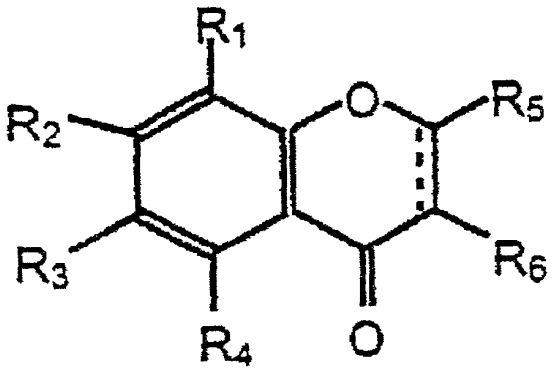
	Dose	Route	Days
• isoflavonoid	100-200 mg/m²/day or 2 - 50 mg/kg/day infusion of 1 h	i.v.	D_{-8}-D_{-1}
• etoposide	1800 mg/m² (infusion of 24 hours)	i.v.	D_{-8}
• thiotepa	300 mg/m²/day as an infusion of 2 hours	i.v.	D_{-7}, D_{-6}, D_{-5}
• cyclo-phosphamide	50 mg/kg/day as an infusion of 1 hour	i.v.	D_{-4}, D_{-3}, D_{-2}, D_{-1}

the bone marrow transplant taking place on D_0.

CLAIMS

1. Composition having an activity on the proliferation of clonogenic cells in tumours and which comprises a therapeutically effective quantity of an isoflavonoid or of an analogue of the chromone type.

2. Composition according to Claim 1, in which the isoflavonoid is chosen from the compounds of formula:



(I)

in which formula:

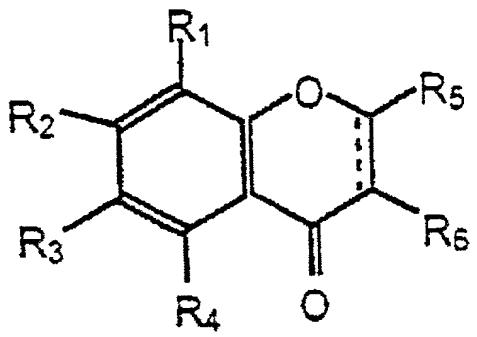
- R_1, R_2, R_3 and R_4 are chosen, independently of each other, from H, OH, a C_1-C_4 alkoxy group, an $-OCOR_7$ group, R_7 being a C_1-C_4 alkyl group, at least one of the substituents R_1, R_2, R_3 or R_4 being other than H and it being possible for R_2 and R_3 to form together a methylenedioxy group,
- R_5 is chosen from H, OH, a C_1-C_4 alkoxy group, an O-glycosyl group and a cyclohexyl group,
- R_6 is chosen from a cyclohexyl group, a phenyl group and a phenyl group substituted 1 to 3 times with groups chosen from H, OH and a C_1-C_4 alkoxy group,
- and —— denotes either a double bond, or a single bond.

3. Composition according to Claim 2, in which the isoflavonoid is chosen from genistein, daidzein and biochanin A.

4. Use of an isoflavonoid or of an analogue of the chromone type for the manufacture of a medicament intended to interfere with the generation of clonogenic

cells in tumours during a treatment of these tumours with at least one cytotoxic agent.

5. Use of a compound chosen from the compounds of formula:



(I)

in which formula:

- R_1, R_2, R_3 and R_4 are chosen, independently of each other, from H, OH, a C_1-C_4 alkoxy group, an $-OCOR_7$ group, R_7 being a C_1-C_4 alkyl group, at least one of the substituents R_1, R_2, R_3 or R_4 being other than H and it being possible for R_2 and R_3 to form together a methylenedioxy group,
- R_5 is chosen from H, OH and a C_1-C_4 alkoxy group, an O-glycosyl group, and a cyclohexyl group,
- R_6 is chosen from a cyclohexyl group, a phenyl group and a phenyl group substituted 1 to 3 times with groups chosen from H, OH and a C_1-C_4 alkoxy group,
- and —— denotes either a double bond, or a single bond,

for the manufacture of a medicament intended to interfere with the generation of clonogenic cells in tumours during a treatment of these tumours with at least one cytotoxic agent.

6. Use according to Claim 5, in which the compound of formula I is chosen from genistein, daidzein and biochanin A.

7. Method for the chemotherapeutic treatment of a tumour in a patient with at least one cytotoxic agent, which comprises the administration, during the treatment with the cytotoxic agent, of a

therapeutically effective quantity of an isoflavonoid or of an analogue of the chromone type.

8. Method according to Claim 7, in which the isoflavonoid or analogue of the chromone type is administered at the beginning of the chemotherapy treatment and at the beginning of each chemotherapy treatment cycle.

DECLARATION AND POWER OF ATTORNEY U.S.A.

ALL PATENTS, INCLUDING DESIGN
FOR APPLICATION BASED ON PCT; PARIS CONVENTION;
NON PRIORITY; OR PROVISIONAL APPLICATIONS

FOR ATTORNEYS' USE ONLY

ATTORNEYS' DOCKET NO.

101 / 102 As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

" Isoflavonoid-based therapeutic composition intended to be used in the treatment of tumours with cytotoxic agents ",

which is described and claimed in:

[X] PCT International Application No. PCT/FR99/01715 filed July 13, 1999

[ ] the attached specification

[ ] the specification in application Serial No. _____ filed _____

(if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.
I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s) (Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed Yes	No
98 09 059	FRANCE	July 15, 1998	[X]	[ ]
			[ ]	[ ]
			[ ]	[ ]

103 / 104 I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application No. _____ Filing Date _____ Application No. _____ Filing Date _____

105 I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith. HARVEY B. JACOBSON, JR. (20,851); D. DOUGLAS PRICE (24,514); JOHN CLARKE HOLMAN (22,769); MARVIN R. STERN (20,640); ALLEN S. MELSER (27,215); MICHAEL R. SLOBASKY (26,421); JONATHAN L. SCHERER (29,851); IRWIN M. AISENBERG (19,007); WILLIAM E. PLAYER (31,409); YOON S. HAM (45,307) and NATHANIEL A. HUMPHRIES (22,772)

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*Inventor(s) name must include at least one unabbreviated first or middle name.

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that th statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the Un States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
Francis DARRO	Robert KISS	Armand FRYDMAN
DATE: December 22, 2000	DATE: December 22, 2000	DATE: December 22, 2000

[ ] Additional inventors are named on separately numbered sheets attached hereto.